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(54) **Title:** BITTER TASTE RECEPTORS

(57) **Abstract:** The present invention relates to novel bitter taste receptors and corresponding agonists as well as processes employing these bitter taste receptors in processes directed at the identification of antagonists and agonists.

Bitter Taste Receptors

Abstract

The present invention relates to bitter-taste receptors and their role in bitter taste transduction. The invention also relates to assays for screening molecules that modulate, e.g. suppress or block bitter taste transduction, or enhance bitter taste response.

Background

Investigators have recently turned their attention to understanding the biological mechanisms of taste, and in particular bitter taste. For a review of the literature see, for example, *Science* **291**, 1557-1560. (2001); *Cell* **100**, 607-610 (2000); *Neuron* **25**, 507-510 (2000); *Nature* **413**, 219-225. (2001); and *J. Biol. Chem.* **277**, 1-4 (2001).

Bitter taste is aversive, and as such provides humans with a mechanism of protection against poisonous substances, which are generally bitter-tasting compounds. More subtly, bitter-tastants also affect the palatability of food, beverages, thereby influencing human nutritional habits as is more fully discussed by Drewnowski in "The Science and Complexity of Bitter Taste", *Nutr. Rev.* **59**, 163-169 (2001). They also affect the palatability of other ingestibles such as orally administered pharmaceuticals and nutraceuticals. Understanding the mechanism of bitter taste transduction has implications for the food and pharmaceutical industries. If the bitter taste transduction pathway can be manipulated, it may be possible to suppress or eliminate bitter taste to render foods more palatable and increase patient compliance in oral pharmaceutics.

Taste transduction involves the interaction of molecules, i.e., tastants with taste receptor-expressing cells which reside in the taste buds located in the papillae of the tongue. Taste buds relay information to the brain on the nutrient content of food and the presence of poisons. Recent advances in biochemical and physiological studies have enabled researchers to conclude that bitter taste transduction is mediated by so-called G-protein coupled receptors (GPCRs). GPCRs are 7 transmembrane domain cell surface proteins that amplify signals generated at a cell surface when the receptor interacts with a ligand (a tastant) whereupon they activate heterotrimeric G-proteins. The G-proteins are protein complexes that are composed of alpha and beta-gamma subunits. They are usually referred to by their

alpha subunits and classified generally into 4 groups: G $_{alpha\ s,\ i,\ q\ and\ 12}$. The G $_{alpha\ q}$ type couple with GPCRs to activate phospholipase C which leads to the increase in cellular Ca^{2+}. There are many G_q-type G-proteins that are promiscuous and can couple to GPCRs, including taste receptors, and these so-called "promiscuous" G-proteins are well known to the man skilled in the art. These G-proteins dissociate into alpha and beta-gamma subunits upon activation, resulting in a complex cascade of cellular events that results in the cell producing cell messengers, such as calcium ions, that enable the cells to send a signal to the brain indicating a bitter response.

There is also anatomical evidence that GPCRs mediate bitter taste transduction: clusters of these receptors are found in mammalian taste cells containing gustducin. Gustducin is a G-protein subunit that is implicated in the perception of bitter taste in mammals, see for example Chandrashekar, J. et al., *Cell* **100**, 703-711 (2000); Matsunami H. et al., *Nature* **404**, 601-604 (2000); or Adler E. et al., *Cell* **100**, 693-702 (2000). cDNAs encoding such GPCRs have been identified, isolated, and used as templates to compare with DNA libraries using *in-silico* data-mining techniques to identify other related receptors. In this manner it has been possible to identify a family of related receptors, the so-called T2R family of receptors, that have been putatively assigned as bitter receptors.

To-date, however, it is not clear as to whether all the bitter taste receptors have been discovered. Further, of those that have been discovered, many have not been matched, or paired, with ligands, and applicant is aware of very few published studies wherein rigorous matching has been undertaken. Chandrashekar, J. et al. in *Cell* **100**, 703-711 (2000), has expressed a human T2R receptor, the so-called hT2R4 receptor, in heterologous systems and looked at the *in vitro* response of this receptor. They found that it provided a response to the bitter compounds denatonium and 6-n-propyl-2-thiouracil. However, the concentrations of bitter tastants needed to activate the hT2R4 receptor were two orders of magnitude higher than the thresholds reported in human taste studies, and so it is not clear that the protein encoded by the hT2R4 gene is a functional bitter receptor. The authors of the Chandrashekar et al. article also looked at a number of mouse T2R receptors with a range of stock bitter-tasting chemicals of disparate chemical structure. However, no study has looked at receptor responses to bitter ligands that are problematic in the food and pharmaceutical industries, and means of suppressing the bitter response to these ligands.

The universe of compounds that provoke a bitter response in humans is structurally very diverse. Therefore, if research into bitter receptors is to be of any practical significance to the food and pharmaceutical industries, all bitter receptors will need to be identified, and once identified, there has to be a rigorous understanding of how specific receptors are matched to particular structural classes of bitter compounds. Unfortunately, although much basic research has been conducted in the area of bitter taste receptors, there are potentially many more bitter receptors to be discovered, and little is still known as to whether the known members of the human T2R family of bitter receptors actually respond to bitter tastants, and if so what, if any, specificity they show to ligand substructures.

Description of the Invention

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention pertains. In case of conflict, the present document, including definitions, will control. Preferred methods and materials are described below, although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention. All publications, patent applications, patents and other references mentioned herein are incorporated by reference in their entirety. The materials, methods, and examples disclosed herein are illustrative only and not intended to be limiting.

Other features and advantages of the invention, e.g., screening for compounds that inhibit bitter taste, will be apparent from the following description, from the drawings and from the claims.

Surprisingly, applicant has now found a new group of putative bitter taste receptors, and in respect of certain known bitter receptors, applicant has found that they respond with specificity towards classes of bitter compounds that are important in the food and pharmaceutical industries.

In a first aspect of the invention there is provided a new group of putative bitter receptors. The genetics of bitter tasting has been extensively studied in mice and rats. Therefore, applicant compared the nucleotide sequences encoding polypeptides previously proposed to be bitter receptors with publicly available human nucleic acid sequences in the NCBI database using the BLAST® search methodology (Parameters: Expect = 0.01, Filter = default).

Surprisingly, the search identified 24 DNA sequences (from human chromosomes 5, 7, and 12) that, because of their homology to a mouse nucleic acid sequence that encodes a polypeptide (T2R5) previously designated as a bitter receptor, we designated as bitter receptor-encoding. Bitter taste receptors were originally assigned identifiers starting with the three characters "T2R" (identifying the receptor family) followed by a number (e.g., 1, 2, 3, etc.) that identifies a particular receptor, e.g., T2R5. More recently a different system has been used in which the identifiers start with five characters "TAS2R" (identifying the receptor family) followed, as previously, by a number (e.g., 1,2, 3, etc.) that identifies a particular receptor, e.g., TAS2R5. A lower case letter in front of the identifier indicates the species of the receptor (e.g., "h" for human, "r" for rat, and "m" for mouse). Thus, for example, mTAS2R5 is a mouse bitter receptor and hTAS2R2 is a human bitter receptor. For consistency the new TAS2R identifier system is used throughout the rest of this application.

Of the 24 coding sequences identified by the search, 12 are believed to be novel; the polypeptides encoded by these novel sequences are designated hTAS2R38-41, and 43-50. The DNA sequences encoding the polypeptides are assigned SEQ ID NOs: 1 (hTAS2R38), 3 (hTAS2R39), 5 (hTAS2R40), 7 (hTAS2R41), 9 (hTAS2R43), 11 (hTAS2R44), 13 (hTAS2R45), 15 (hTAS2R46), 17 (hTAS2R47), 19 (hTAS2R48), 21 (hTAS2R49), and 23 (hTAS2R50), respectively, and the amino acid sequences of the polypeptides are assigned SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24, respectively.

The 12 additional (possibly novel) sequences identified by the search encode polypeptides, which are designated hTAS2R1, 4, 5, 7-10, 13, 14, 16, 3, 42 and 60. The DNA sequences encoding the polypeptides are assigned SEQ ID NOs: 25 (hTAS2R1), 27 (hTAS2R4), 29 (hTAS2R5), 31 (hTAS2R7), 33 (hTAS2R8), 35 (hTAS2R9), 37 (hTAS2R10), 39 (hTAS2R13), 41 (hTAS2R14), 43 (hTAS2R16), 45 (hTAS2R3), 47 (hTAS2R42), and 49 (hTAS2R60), respectively, and the amino acid sequences of the polypeptides are assigned SEQ ID NOs: 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48 and 50, respectively.

Thus, one aspect of the present invention is a polynucleotide selected from the group consisting of

(a) polynucleotides encoding at least the mature form of the polypeptide having the deduced amino acid sequence as shown in SEQ ID NOs 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24;

(b) polynucleotides having the coding sequence, as shown in SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 23 encoding at least the mature form of the polypeptide;

(c) polynucleotides encoding a fragment or derivative of a polypeptide encoded by a polynucleotide of any one of (a) to (b), wherein in said derivative one or more amino acid residues are conservatively substituted compared to said polypeptide, and said fragment or derivative has bitter substance binding activity;

(d) polynucleotides which are at least 50% identical to a polynucleotide as defined in any one of (a) to (c) and which code for a polypeptide having bitter substance binding activity; and

(e) polynucleotides the complementary strand of which hybridizes, preferably under stringent conditions to a polynucleotide as defined in any one of (a) to (d) and which code for a polypeptide having bitter substance binding activity;

or the complementary strand of such a polynucleotide.

A polypeptide that exhibits bitter substance binding activity is a polypeptide that has at least 20% (e.g., at least: 20%; 30%; 40%; 50%; 60%; 70%; 80%; 90%; 95%; 98%; 99%; 99.5%; or 100% or even more) of the ability of the respective full-length TAS2R to bind to a given bitter substance. Binding assays and bitter substances are described herein below.

In a preferred embodiment the polynucleotide of the present invention encodes a polypeptide that still exhibits essentially the same activity as the respective mature bitter taste receptor, i.e. has "bitter taste receptor activity". Preferably the polypeptide has at least 20% (e.g., at least: 20%; 30%; 40%; 50%; 60%; 70%; 80%; 90%; 95%; 98%; 99%; 99.5%; or 100% or even more) of the ability of the respective full-length TAS2R to release intracellular calcium in a heterologous cell expression system like, for example, HEK293/15 – cells, which stably express the alpha-subunit of promiscuous G-proteins, e.g. the mouse G_{15} subunit, in response to bitter tastants, which is dependent on the expression of polypeptides encoded by the polynucleotides of the present invention. The amount of intracellular calcium release can be monitored by, for example, the *in vitro* FLIPR assay described herein below.

The TAS2R nucleic acid molecules of the invention can be DNA, cDNA, genomic DNA, synthetic DNA, or, RNA, and can be double-stranded or single-stranded, the sense and/or an antisense strand. Segments of these molecules are also considered within the scope of

the invention, and can be produced by, for example, the polymerase chain reaction (PCR) or generated by treatment with one or more restriction endonucleases. A ribonucleic acid (RNA) molecule can be produced by *in vitro* transcription.

The polynucleotide molecules of the invention can contain naturally occurring sequences, or sequences that differ from those that occur naturally, but, due to the degeneracy of the genetic code, encode the same polypeptide (for example, the polypeptides with SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24). In addition, these nucleic acid molecules are not limited to coding sequences, e.g., they can include some or all of the non-coding sequences that lie upstream or downstream from a coding sequence.

The polynucleotide molecules of the invention can be synthesized *in vitro* (for example, by phosphoramidite-based synthesis) or obtained from a cell, such as the cell of a bacteria mammal. The nucleic acids can be those of a human but also derived from a non-human primate, mouse, rat, guinea pig, cow, sheep, horse, pig, rabbit, dog, or cat as long as they fulfill the criteria set out above. Combinations or modifications of the nucleotides within these types of nucleic acids are also encompassed.

In addition, the isolated nucleic acid molecules of the invention encompass segments that are not found as such in the natural state. Thus, the invention encompasses recombinant nucleic acid molecules incorporated into a vector (for example, a plasmid or viral vector) or into the genome of a heterologous cell (or the genome of a homologous cell, at a position other than the natural chromosomal location). Recombinant nucleic acid molecules and uses therefore are discussed further below.

A polynucleotide belonging to a family of any of the TAS2R disclosed herein or a protein can be identified based on its similarity to the relevant TAS2R gene or protein, respectively. For example, the identification can be based on sequence identity. In certain preferred embodiments the invention features isolated nucleic acid molecules which are at least 50% (or 55%, 65%, 75%, 85°/a, 95%, or 98%) identical to: (a) a nucleic acid molecule that encodes the polypeptide of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, or 24; (b) the nucleotide sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23; and (c) a nucleic acid molecule which includes a segment of at least 30 (e.g., at least 30, 40, 50, 60, 80, 100, 125, 150, 175, 200, 250, 300, 400, 500, 600, 700; 800, 850, 900, 950,

1000, or 1010) nucleotides of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, or 23.

The determination of percent identity between two sequences is accomplished using the mathematical algorithm of Karlin and Altschul, *Proc. Natl. Acad. Sci. USA* **90**, 5873-5877, 1993. Such an algorithm is incorporated into the BLASTN and BLASTP programs of Altschul et al. (1990) J. Mol. Biol. 215, 403-410. BLAST nucleotide searches are performed with the BLASTN program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to HIN-1-encoding nucleic acids. BLAST protein searches are performed with the BLASTP program, score = 50, wordlength = 3, to obtain amino acid sequences homologous to the TAS2R polypeptide. To obtain gapped alignments for comparative purposes, Gapped BLAST is utilized as described in Altschul et al. (1997) Nucleic Acids Res. 25, 3389-3402. When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs.

Hybridization can also be used as a measure of homology between two nucleic acid sequences. A nucleic acid sequence encoding any of the TAS2R disclosed herein, or a portion thereof, can be used as a hybridization probe according to standard hybridization techniques. The hybridization of a TAS2R probe to DNA or RNA from a test source (e.g., a mammalian cell) is an indication of the presence of the relevant TAS2R DNA or RNA in the test source. Hybridization conditions are known to those skilled in the art and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N.Y., 6.3.1-6.3.6, 1991. Moderate hybridization conditions are defined as equivalent to hybridization in 2X sodium chloride/sodium citrate (SSC) at 30°C, followed by a wash in 1 X SSC, 0.1% SDS at 50°C. Highly stringent conditions are defined as equivalent to hybridization in 6X sodium chloride/sodium citrate (SSC) at 45°C, followed by a wash in 0.2 X SSC, 0.1 % SDS at 65°C.

An "isolated DNA" is either (1) a DNA that contains sequence not identical to that of any naturally occurring sequence, or (2), in the context of a DNA with a naturally-occurring sequence (e.g., a cDNA or genomic DNA), a DNA free of at least one of the genes that flank the gene containing the DNA of interest in the genome of the organism in which the gene containing the DNA of interest naturally occurs. The term therefore includes a recombinant DNA incorporated into a vector, into an autonomously replicating plasmid or virus, or into the genomic DNA of a prokaryote or eukaryote. The term also includes a

separate molecule such as a cDNA where the corresponding genomic DNA has introns and therefore a different sequence; a genomic fragment that lacks at least one of the flanking genes; a fragment of cDNA or genomic DNA produced by polymerase chain reaction (PCR) and that lacks at least one of the flanking genes; a restriction fragment that lacks at least one of the flanking genes; a DNA encoding a non-naturally occurring protein such as a fusion protein, mutein, or fragment of a given protein; and a nucleic acid which is a degenerate variant of a cDNA or a naturally occurring nucleic acid. In addition, it includes a recombinant nucleotide sequence that is part of a hybrid gene, i.e., a gene encoding a non-naturally occurring fusion protein. It will be apparent from the foregoing that isolated DNA does not mean a DNA present among hundreds to millions of other DNA molecules within, for example, cDNA or genomic DNA libraries or genomic DNA restriction digests in, for example, a restriction digest reaction mixture or an electrophoretic gel slice.

A further aspect of the present invention is a vector containing the polynucleotide(s) of the present invention or a protein encoded by a polynucleotide of the present invention The term "vector" refers to a protein or a polynucleotide or a mixture thereof which is capable of being introduced or of introducing the proteins and/or nucleic acid comprised into a cell. It is preferred that the proteins encoded by the introduced polynucleotide are expressed within the cell upon introduction of the vector.

In a preferred embodiment the vector of the present invention comprises plasmids, phagemids, phages, cosmids, artificial mammalian chromosomes, knock-out or knock-in constructs, viruses, in particular adenoviruses, vaccinia viruses, attenuated vaccinia viruses, canary pox viruses, lentivirus (Chang, L.J. and Gay, E.E. (20001) Curr. Gene Therap. 1:237-251), herpes viruses, in particular Herpes simplex virus (HSV-1, Carlezon, W.A. et al. (2000) Crit. Rev. Neurobiol.), baculovirus, retrovirus, adeno-associated-virus (AAV, Carter, P.J. and Samulski, R.J. (2000) J. Mol. Med. 6:17-27), rhinovirus, human immune deficiency virus (HIV), filovirus and engineered versions thereof (see, for example, Cobinger G. P. et al (2001) Nat. Biotechnol. 19:225-30), virosomes, "naked" DNA liposomes, and nucleic acid coated particles, in particular gold spheres. Particularly preferred are viral vectors like adenoviral vectors or retroviral vectors (Lindemann et al. (1997) Mol. Med. 3:466-76 and Springer et al. (1998) Mol. Cell. 2:549-58). Liposomes are usually small unilamellar or multilamellar vesicles made of cationic, neutral and/or anionic lipids, for example, by ultrasound treatment of liposomal suspensions. The DNA can, for

example, be ionically bound to the surface of the liposomes or internally enclosed in the liposome. Suitable lipid mixtures are known in the art and comprise, for example, DOTMA (1, 2-Dioleyloxpropyl-3-trimethylammoniumbromid) and DPOE (Dioleoylphosphatidyl-ethanolamin) which both have been used on a variety of cell lines.

Nucleic acid coated particles are another means for the introduction of nucleic acids into cells using so called "gene guns", which allow the mechanical introduction of particles into the cells. Preferably the particles itself are inert, and therefore, are in a preferred embodiment made out of gold spheres.

In a further aspect the polynucleotide of the present invention is operatively linked to expression control sequences allowing expression in prokaryotic and/or eukaryotic host cells. The transcriptional/translational regulatory elements referred to above include but are not limited to inducible and non-inducible, constitutive, cell cycle regulated, metabolically regulated promoters, enhancers, operators, silencers, repressors and other elements that are known to those skilled in the art and that drive or otherwise regulate gene expression. Such regulatory elements include but are not limited to regulatory elements directing constitutive expression like, for example, promoters transcribed by RNA polymerase III like , e.g., promoters for the snRNA U6 or scRNA 7SK gene, the cytomegalovirus hCMV immediate early gene, the early or late promoters of SV40 adenovirus, viral promoter and activator sequences derived from, e.g. , NBV, HCV, HSV, HPV, EBV, HTLV, MMTV or HIV; which allow inducible expression like, for example, CUP-1 promoter, the tet-repressor as employed, for example, in the tet-on or tet-off systems, the lac system, the trp system; regulatory elements directing tissue specific expression, preferably taste bud specific expression, e.g., PLCβ2 promoter or gustducin promoter, regulatory elements directing cell cycle specific expression like, for example, cdc2, cdc25C or cyclin A; or the TAC system, the TRC system, the major operator and promoter regions of phage A, the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase, the promoters of acid phosphatase, and the promoters of the yeast α- or a-mating factors.

As used herein, "operatively linked" means incorporated into a genetic construct so that expression control sequences effectively control expression of a coding sequence of interest.

Similarly, the polynucleotides of the present invention can form part of a hybrid gene encoding additional polypeptide sequences, for example, a sequence that functions as a marker or reporter. Examples of marker and reporter genes include β-lactamase, chloramphenicol acetyltransferase (CAT), adenosine deaminase (ADA), aminoglycoside phosphotransferase (neo^r, $G418^r$), dihydrofolate reductase (DHFR), hygromycin-B-phosphotransferase (HPH), thymidine kinase (TK), lacZ (encoding β-galactosidase), and xanthine guanine phosphoribosyltransferase (XGPRT). As with many of the standard procedures associated with the practice of the invention, skilled artisans will be aware of additional useful reagents, for example, additional sequences that can serve the function of a marker or reporter. Generally, the hybrid polypeptide will include a first portion and a second portion; the first portion being a TAS2R polypeptide and the second portion being, for example, the reporter described above or an Ig constant region or part of an Ig constant region, e.g., the CH2 and CH3 domains of IgG2a heavy chain. Other hybrids could include an antigenic tag or His tag to facilitate purification and/or detection. Recombinant nucleic acid molecules can also contain a polynucleotide sequence encoding a TAS2R polypeptide operatively linked to a heterologous signal sequence. Such signal sequences can direct the protein to different compartments within the cell and are well known to someone of skill in the art. A preferred signal sequence is a sequence that facilitates secretion of the resulting protein.

Another aspect of the present invention is a host cell genetically engineered with the polynucleotide or the vector as outlined above. The host cells that may be used for purposes of the invention include but are not limited to prokaryotic cells such as bacteria (for example, *E. coli* and *B. subtilis*), which can be transformed with, for example, recombinant bacteriophage DNA, plasmid DNA, or cosmid DNA expression vectors containing the polynucleotide molecules of the invention; simple eukaryotic cells like yeast (for example, *Saccharomyces* and *Pichia*), which can be transformed with, for example, recombinant yeast expression vectors containing the polynucleotide molecule of the invention; insect cell systems like, for example, Sf9 of Hi5 cells, which can be infected with, for example, recombinant virus expression vectors (for example, baculovirus) containing the polynucleotide molecules of the invention; Xenopus oocytes, which can be injected with, for example, plasmids; plant cell systems, which can be infected with, for example, recombinant virus expression vectors (for example, cauliflower mosaic virus (CaMV) or tobacco mosaic virus (TMV)) or transformed with recombinant plasmid expression vectors (for example, Ti

plasmid) containing a TAS2R nucleotide sequence; or mammalian cell systems (for example, COS, CHO, BHK, HEK293, VERO, HeLa, MDCK, Wi38, and NIH 3T3 cells), which can be transformed with recombinant expression constructs containing, for example, promoters derived, for example, from the genome of mammalian cells (for example, the metallothionein promoter) from mammalian viruses (for example, the adenovirus late promoter and the vaccinia virus 7.5K promoter) or from bacterial cells (for example, the tet-repressor binding its employed in the tet-on and tet-off systems). Also useful as host cells are primary or secondary cells obtained directly from a mammal and transfected with a plasmid vector or infected with a viral vector. Depending on the host cell and the respective vector used to introduce the polynucleotide of the invention the polynucleotide can integrate, for example, into the chromosome or the mitochondrial DNA or can be maintained extrachromosomally like, for example, episomally or can be only transiently comprised in the cells.

In a preferred embodiment, the TAS2R encoded by the polynucleotides of the present invention and which are expressed by such cells are functional, i.e., upon binding to one or more bitter molecules they trigger an activation pathway in the cell. The cells are preferably mammalian (e.g., human, non-human primate, horse, bovine, sheep, goat, pig, dog, cat, goat, rabbit, mouse, rat, guinea pig, hamster, or gerbil) cells, insect cells, bacterial cells, or fungal (including yeast) cells.

A further aspect of the present invention is a transgenic non-human animal containing a polynucleotide, a vector and/or a host cell as described above. The animal can be a mosaic animal, which means that only part of the cells making up the body comprise polynucleotides, vectors, and/or cells of the present invention or the animal can be a transgenic animal which means that all cells of the animal comprise the polynucleotides and/or vectors of the present invention or are derived from a cell of the present invention. Mosaic or transgenic animals can be either homo- or heterozygous with respect to the polynucleotides of the present invention contained in the cell. In a preferred embodiment the transgenic animals are either homo- or heterozygous knock-out or knock-in animals with respect to the genes which code for the proteins of the present invention. The animals can in principal be any animal, preferably, however, it is a mammal, selected from the group of non-human pimate horse, bovine, sheep, goat, pig, dog, cat, goat, rabbit, mouse, rat, guinea pig, hamster, or gerbil.

Another aspect of the present invention is a process for producing a polypeptide encoded by a polynucleotide of the present invention comprising: culturing the host cell described above and recovering the polypeptide encoded by said polynucleotide. Preferred combinations of host cells and vectors are outlined above and further combination will be readily apparent to someone of skill in the art. Depending on the intended later use of the recovered peptide a suitable cell type can be chosen. Eukaryotic cells are preferably chosen, if it is desired that the proteins produced by the cells exhibit an essentially natural pattern of glycosylation and prokaryotic cells are chosen, if, for example, glycosylation or other modifications, which are normally introduced into proteins only in eukaryotic cells, are not desired or not needed.

A further aspect of the invention is a process for producing cells capable of expressing at least one of the bitter taste receptor polypeptides comprising genetically engineering cells *in vitro* with at least one of the vectors described above, wherein said bitter taste receptor polypeptide(s) is(are) encoded by a polynucleotide of the present invention.

Another aspect of the invention is a polypeptide having the amino acid sequence encoded by a polynucleotide of the invention or obtainable by the process mentioned above. The polypeptides of the invention include all those disclosed herein and functional fragments of these polypeptides. "Polypeptide" and "protein" are used interchangeably and mean any peptide-linked chain of amino acids, regardless of length or posttranslational modification. As used herein, a functional fragment of a TAS2R is a fragment of the TAS2R that is shorter than the full-length TAS2R but that has at least 20% (e.g., at least: 20%; 30%; 40%; 50%; 60%; 70%; 80%; 90%; 95%; 98%; 99%; 99.5%; or 100% or even more) of the ability of the full-length TAS2R to bind to a bitter substance to which the full-length TAS2R binds. Binding assays and bitter substances are described herein. Further bitter substances can be identified by the binding assays and bitter taste receptor activity assays described herein. The polypeptides embraced by the invention also include fusion proteins that contain either a full-length TAS2R polypeptide or a functional fragment of it fused to an unrelated amino acid sequence. The unrelated sequences can be additional functional domains or signal peptides. Signal peptides are described in greater detail and exemplified below.

The polypeptides can be any of those described above but with not more than 50 (e.g., not more than: 50, 45, 40, 35, 30, 25, 20, 15, 14, 13, 12, 11, 10, nine, eight, seven, six, five, four, three, two, or one) conservative substitutions. Conservative substitutions typically include substitutions within the following groups: glycine and alanine; valine, isoleucine, and leucine; aspartic acid and glutamic acid; asparagine, glutamine, serine and threonine; lysine, histidine and arginine; and phenylalanine and tyrosine. All that is required of a polypeptide having one or more conservative substitutions is that it has at least 20% (e.g., at least: 20%; 30%; 40%; 50%; 60%; 70%; 80%; 90%; 95%; 98%; 99%; 99.5%; or 100% or even more) of the ability of the wild-type, full-length TAS2R to bind to a bitter substance, preferably the ability to release intracellular calcium, when expressed in a cellular system.

The polypeptides can be purified from natural sources (e.g., blood, serum, plasma, tissues or cells such as normal tongue cells or any cell that naturally produces the relevant TAS2R polypeptides). Smaller peptides (less than 50 amino acids long) can also be conveniently synthesized by standard chemical means. In addition, both polypeptides and peptides can be produced by standard *in vitro* recombinant DNA techniques and *in vivo* transgenesis, using nucleotide sequences encoding the appropriate polypeptides or peptides. Methods well-known to those skilled in the art can be used to construct expression vectors containing relevant coding sequences and appropriate transcriptional/translational control signals. See, for example, the techniques described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* (2nd Ed.) [Cold Spring Harbor Laboratory, N.Y., 1989], and Ausubel et al., *Current Protocols in Molecular Biology* [Green Publishing Associates and Wiley Interscience, N.Y., 1989].

Polypeptides and fragments of the invention also include those described above, but modified for *in vivo* use by the addition, at the amino- and/or carboxyl-terminal ends, of blocking agents to facilitate survival of the relevant polypeptide *in vivo*. This can be useful in those situations in which the peptide termini tend to be degraded by proteases prior to cellular uptake. Such blocking agents can include, without limitation, additional related or unrelated peptide sequences that can be attached to the amino and/or carboxyl terminal residues of the peptide to be administered. This can be done either chemically during the synthesis of the peptide or by recombinant DNA technology by methods familiar to artisans of average skill.

Alternatively, blocking agents such as pyroglutamic acid or other molecules known in the art can be attached to the amino and/or carboxyl terminal residues, or the amino group at the amino terminus or carboxyl group at the carboxyl terminus can be replaced with a different moiety. Likewise, the peptides can be covalently or noncovalently coupled to pharmaceutically acceptable "carrier" proteins prior to administration.

Also of interest are peptidomimetic compounds that are designed based upon the amino acid sequences of the functional peptides or peptide fragments. Peptidomimetic compounds are synthetic compounds having a three-dimensional conformation (i.e., a "peptide motif") that is substantially the same as the three-dimensional conformation of a selected peptide. The peptide motif provides the peptidomimetic compound with the ability to bind to a bitter compound in a manner qualitatively identical to that of the TAS2R functional fragment from which the peptidomimetic was derived. Peptidomimetic compounds can have additional characteristics that enhance their therapeutic utility, such as increased cell permeability and prolonged biological half-life.

The peptidomimetics typically have a backbone that is partially or completely nonpeptide, but with side groups that are identical to the side groups of the amino acid residues that occur in the peptide on which the peptidomimetic is based. Several types of chemical bonds, e.g., ester, thioester, thioamide, retroamide, reduced carbonyl, dimethylene and ketomethylene bonds, are known in the art to be generally useful substitutes for peptide bonds in the construction of protease-resistant peptidomimetics.

The term "isolated" polypeptide or peptide fragment as used herein refers to a polypeptide or a peptide fragment which either has no naturally-occurring counterpart or has been separated or purified from components which naturally accompany it, e.g., in tissues such as tongue, pancreas, liver, spleen, ovary, testis, muscle, joint tissue, neural tissue, gastrointestinal tissue or tumor tissue, or body fluids such as blood, serum, or urine. Typically, the polypeptide or peptide fragment is considered "isolated" when it is at least 70%, by dry weight, free from the proteins and other naturally-occurring organic molecules with which it is naturally associated. Preferably, a preparation of a polypeptide (or peptide fragment thereof) of the invention is at least 80%, more preferably at least 90%, and most preferably at least 99%, by dry weight, the polypeptide (or the peptide fragment thereof), respectively,

of the invention. Thus, for example, a preparation of polypeptide x is at least 80%, more preferably at least 90%, and most preferably at least 99%, by dry weight, polypeptide x. Since a polypeptide that is chemically synthesized is, by its nature, separated from the components that naturally accompany it, the synthetic polypeptide is "isolated."

An isolated polypeptide (or peptide fragment) of the invention can be obtained, for example, by extraction from a natural source (e.g., from tissues or bodily fluids); by expression of a recombinant nucleic acid encoding the polypeptide; or by chemical synthesis. A polypeptide that is produced in a cellular system different from the source from which it naturally originates is "isolated," because it will necessarily be free of components which naturally accompany it. The degree of isolation or purity can be measured by any appropriate method, e.g., column chromatography, polyacrylamide gel electrophoresis, or HPLC analysis.

A further aspect of the invention is an antibody, which specifically binds to the polypeptide encoded by a polynucleotide of the invention or obtainable by the process mentioned above. The term "antibody" comprises monoclonal and polyclonal antibodies and binding fragments thereof, in particular Fc-fragments as well as so called "single-chain-antibodies" (Bird R. E. et al (1988) Science 242:423-6), chimeric, humanized, in particular CDR-grafted antibodies, and dia or tetrabodies (Holliger P. et al (1993) Proc. Natl. Acad. Sci. U.S.A. 90:6444-8). Also comprised are immunoglobulin like proteins that are selected through techniques including, for example, phage display to specifically bind to the polypeptides of the present invention. Preferred antibodies bind to the extracellular domain of bitter receptors and in particular to those domains responsible for binding to bitter tastants.

In yet another embodiment there is provided a molecule, or collections of molecules containing a molecule, that act to antagonise aforementioned receptors in particular the bitter taste response, and methods for screening for such molecules.

Therefore, a further aspect of the invention is a nucleic acid molecule which specifically hybridizes to a polynucleotide of the present invention. In particular this nucleic acid molecule is an inhibiting RNA. Preferred inhibiting RNAs are antisense constructs hybridizing to a polynucleotide of the present invention, RNAi, siRNA or a ribozyme. The design of such inhibiting RNAs would be readily apparent to someone of skill in the art.

Another type of antagonist/inhibitor against the polypeptides of the present invention is an antibody, which is preferably directed against the extracellular domain of the respective bitter taste receptor and even more preferably binds to the site(s) of the receptor that interact(s) with the bitter substance(s) essentially without triggering the release of intracellular calcium. Further antagonists to the bitter taste response of a receptor are fragments of the receptor which have the capability to bind to the bitter substances as defined above. Such fragments can bind to the bitter substance and, thus, competitively antagonize the activity of the respective TAS2R. If such antagonists are, for example, employed within foodstuff to suppress the bitter taste of a specific bitter substance they might be exposed to a proteolytic environment and in this case the modifications of the polypeptides outlined above could be used to stabilize the competitive bitter receptor antagonist. However, various additional modifications, which stabilize such fragments will be readily apparent to the skilled person.

Antagonists and agonists of the bitter taste receptors described herein are of great importance for specific stimulation of a given bitter taste receptor or to antagonize it. The bitter taste response of the receptor is elicited by the specific binding of the respective bitter substance. Therefore, the present invention is also directed at a process for isolating a compound that binds to a polypeptide encoded by a polynucleotide selected from the group consisting of:

(a) polynucleotides encoding at least the mature form of the polypeptide having the deduced amino acid sequence as shown in SEQ ID NOs 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48 and 50;

(b) polynucleotides having the coding sequence, as shown in SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47 and 49 encoding at least the mature form of the polypeptide;

(c) polynucleotides encoding a fragment or derivative of a polypeptide encoded by a polynucleotide of any one of (a) to (b), wherein in said derivative one or more amino acid residues are conservatively substituted compared to said polypeptide, and said fragment or derivative has bitter substance binding activity;

(d) polynucleotides which are at least 50% identical to a polynucleotide as defined in any one of (a) to (c) and which code for a polypeptide having bitter substance binding activity; and

(e) polynucleotides the complementary strand of which hybridizes, preferably under stringent conditions to a polynucleotide as defined in any one of (a) to (d) and which code for a polypeptide having bitter substance binding activity;

comprising:

(1) contacting said polypeptide or a host cell genetically engineered with said polynucleotide or with a vector containing said polynucleotide with a compound;
(2) detecting the presence of the compound which binds to said polypeptide; and
(3) determining whether the compound binds said polypeptide.

A polynucleotide employed in this process is in preferred embodiments of the invention at least 50% (or 55%, 65%, 75%, 85°/a, 95%, or 98%) identical to: (a) a nucleic acid molecule that encodes the polypeptide of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48 or 50; (b) the nucleotide sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47 or 49; and has a length of at least 30 (e.g., at least 30, 40, 50, 60, 80, 100, 125, 150, 175, 200, 250, 300, 400, 500, 600, 700, 800, 850, 900, 950, 1000, or 1010) of the nucleotides of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, or 49.

Furthermore for all of the above described hTAS2Rs, which can be employed in a process for isolating binding compounds, with the exception of hTAS2R40, single nucleotide polymorphisms are known. 79 of these are listed in Table I below. 61 of those result in an amino acid change. Polynucleotides or polypeptides that differ from the respectively in SEQ ID 1-50 indicated sequences by the nucleotide and amino acid change as indicted in Table I can similarly be employed for the process of the present invention.

Table I

Gen + Acession No.	Name of SNP	Substitution		Position		Allelic frequency
		Base	Amino acid	Base pair	Amino acid	
hTAS2R1 NM019599	rs2234231	C/T	P/L	128	43	unknown

	rs41469	G/A	R/H	332	111	A 0.46/G 0.54
	rs223432	G/A	C/Y	422	141	unknown
	rs2234233	C/T	R/W	616	206	C 0.87/T 0.13
	rs2234234	C/T	S/S	675	225	unknown
	rs2234235	T/C	L/L	850	284	unknown
hTAS2R3 NM016943	rs227009	C/T	G/G	807	369	unknown
hTAS2R4	ss3181498	G/A	R/Q	8	3	unknown
NM016944	rs2233996	G/C	R/R	9	3	unknown
	rs2233997	A/C	Y/C	17	6	unknown
	rs2233998	T/C	F/S	20	7	unknown
	rs2233999	T/A	F/L	186	62	unknown
	rs2234000	C/T	T/M	221	74	C 0.94/T 0.56
	rs2234001	G/C	V/L	286	96	C 0.78/G 0.22
	rs2234002	G/A	S/N	512	171	A 0.78/ G 0.22
	rs2234003	A/G	I/V	571	191	unknown
hTAS2R5	rs2234013	G/A	G/S	58	20	unknown
NM018980	rs2227264	G/T	S/I	77	26	unknown
	rs2234014	C/T	P/L	338	113	unknown
	rs2234015	G/A	R/Q	638	213	unknown
	rs2234016	G/T	R/L	294	881	unknown
hTAS2R7 NM023919	rs3759251	A/T	T/S	787	263	A 0.97/T 0.03
	rs3759252	C/A	I/I	828	276	unknown
	rs619381	G/A	M/I	912	304	unknown
hTAS2R8	ss2391467	G/A	L/L	549	183	unknown
NM023918	rs2537817	A/G	M/V	922	308	unknown
hTAS2R9	rs3741845	T/C	V/A	560	187	C 073/T 027
NM23917	rs3944035	C/T	L/F	910	304	unknown
	rs2159903	C/T	P/L	926	309	unknown
hTAS2R10 NM23921	rs597468	C/T	T/M	467	156	unknown
hTAS2R13 NM23920	ss1478988	A/G	N/S	776	259	C 0.73/T 0.27
hTAS2R14 NM23922	rs3741843	G/A	R/R	375	125	A 0.97/G 0.03
hTAS2R16	rs2233988	C/T	T/T	300	100	unknown
NM016945	rs2692396	G/C	V/V	303	101	unknown
	rs2233989	T/C	L/L	460	154	unknown
	rs846664	T/G	N/K	516	172	A 0.71/C 0.29
	rs860170	G/A	R/H	665	222	A 0.55/G 0.45
hTAS2R38 AF494321	PTC Paper	G/A	V/I	886	296	G 0.38/A 0.62
	rs1726866	T/C	V/A	785	262	G 0.38/T 0.62
	rs713598	C/T	A/P	49	145	C 0.36/G 0.64
	hTAS2R38 SNP1	A/T	N/I	557	186	C 0.60/G 0.40
hTAS2R39 AF494230	hTAS2R39 SNP1	A/AA	frameshift	967	323	unknown
hTAS2R41	rs1404635	A/G	T/T	189	64	unknown
AF494232	hTAS2R41 SNP1	T/C	L/P	380	127	unknown
	hTAS2R41 SNP2	A/G	S/S	885	295	unknown
hTAS2R42	rs1650017	G/C	A/P	931	311	unknown
AX097739	rs1669411	T/C	N/N	930	310	unknown
	rs1669412	G/A	R/Q	875	292	unknown
	rs1451772	A/G	Y/C	794	265	unknown
	rs1669413	G/T	G/W	763	255	unknown
	rs1650019	A/G	L/L	561	187	unknown
hTAS2R43	rs3759246	G/C	R/T	893	298	unknown
AF494237	hTAS2R43 SNP1	C/G	S/W	104	35	unknown
	hTAS2R43 SNP2	G/A	R/H	635	212	unknown
	hTAS2R43 SNP3	G/C	T/T	663	221	unknown
hTAS2R44	rs3759247	G/A	W/ stop	900	300	unknown
AF494228	rs3759246	G/C	R/T	893	298	unknown
	hTAS2R44 SNP1	A/T	M/L	162	484	unknown
	hTAS2R44 SNP2	T/A	F/Y	869	290	unknown
	hTAS2R44 SNP3	G/A	V/M	899	297	unknown
hTAS2R45 AF494226	rs3759247	A/G	G/stop	900	300	unknown

	rs3759246	G/C	R/T	893	298	unknown
	rs3759245	C/T	R/C	712	238	unknown
	rs3759244	T/C	F/L	703	235	unknown
hTAS2R46	rs2708381	G/A	W/stop	749	250	unknown
AF494227	rs2708380	T/A	L/M	682	228	unknown
	rs2598002	T/G	F/V	106	36	unknown
	hTAS2R46 SNP1	A/T	Q/H	888	296	unknown
	hTAS2R46 SNP2	A/G	M/V	889	297	unknown
	hTAS2R46 SNP3	T/C	F/F	108	36	unknown
hTAS2R47	rs2597924	G/A	R/H	920	307	unknown
AF494233	rs1669405	T/G	L/W	842	281	unknown
	rs2599404	T/G	F/L	756	252	unknown
	rs2600355	T/G	V/V	54	18	unknown
hTAS2R48 AF494234	rs1868769	T/C	L/L	418	140	unknown
hTAS2R49 AF494236	hTAS2R49 SNP1	A/G	K/R	164	55	unknown
hTAS2R50 AF494235	rs1376521	A/G	Y/C	608	203	G 0.66/ A0.34
	hTAS2R50 SNP1	A/G	P/P	777	259	unknown

A polypeptide that exhibits bitter substance binding activity is a polypeptide that has at least 20% (e.g., at least: 20%; 30%; 40%; 50%; 60%; 70%; 80%; 90%; 95%; 98%; 99%; 99.5%; or 100% or even more) of the ability of the respective full-length TAS2R to bind to a given bitter substance. Binding assays and bitter substances are described herein.

The term "contacting" in the context of the present invention means any interaction between the compound with the polypeptide of the invention, whereby any of the at least two components can be independently of each other in a liquid phase, for example in solution, or in suspension or can be bound to a solid phase, for example, in the form of an essentially planar surface or in the form of particles, pearls or the like. In a preferred embodiment a multitude of different compounds are immobilized on a solid surface like, for example, on a compound library chip and the protein of the present invention is subsequently contacted with such a chip. In another preferred embodiment the cells genetically engineered with the polynucleotide of the invention or with a vector containing such a polynucleotide express the bitter taste receptor at the cell surface and are contacted separatley in small containers, e. g., microtitre plates, with various compounds.

Detecting the presence and the binding of the compound to the polypeptide can be carried out, for example, by measuring a marker that can be attached either to the protein or to the compound. Suitable markers are known in the art and comprise, for example, fluorescence, enzymatic or radioactive markers. The binding of the two components can, however, also be measured by the change of an electrochemical parameter of the binding compound or of the protein, e.g. a change of the redox properties of either the protein or the binding compound, upon binding. Suitable methods of detecting such changes comprise, for example,

potentiometric methods. Further methods for detecting and/or measuring the binding of the two components to each other are known in the art and can without limitation also be used to measure the binding of the compound to the polypeptide. The effect of the binding of the compound on the activity of the polypeptide can also be measured by assessing changes in the cells that express the polypeptides, for example, by assaying the intracellular release of calcium upon binding of the compound.

As a further step after measuring the binding of a compound and after having measured the binding strength of at least two different compounds at least one compound can be selected, for example, on grounds of a higher binding strength or on grounds of the detected intracellular release of calcium.

The thus selected compound is than in a preferred embodiment modified in a further step. Modification can be effected by a variety of methods known in the art, which include without limitation the introduction of one or more, preferably two, three or four novel side chains or residues or the exchange of one or more functional groups like, for example, introduction or exchange of halogens, in particular F, Cl or Br; the introduction or exchange of lower alkyl residues, preferably having one to five carbon atoms like, for example, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, tert-butyl, n-pentyl or iso-pentyl residues; lower alkenyl residues, preferably having two, three, four or five carbon atoms; lower alkinyl residues, preferably having two, three, four or five carbon atoms, which can in a preferred embodiment be further substituted with F, Cl, Br, NH_2, NO_2, OH, SH, NH, CN, aryl, heteroaryl, COH or COOH group; or the introduction of, for example, one or more residue(s) selected from the group consisting of NH_2, NO_2, OH, SH, NH, CN, aryl, alkylaryl, heteroaryl, akylheteroaryl, COH or COOH group.

The thus modified binding substances are than individually tested with the method of the present invention, i.e. they are contacted with the polypeptide as such or with the polypeptide expressed in a cell, and subsequently binding of the modified compounds is measured. In this step both the binding *per se* can be measured and/or the effect of the function of the protein like, e.g. the intracellular calcium release. If needed the steps of selecting the compound, modifying the compound, contacting the compound with a polypeptide of the invention and measuring the binding of the modified compound to the polypeptide can be repeated a third or any given number of times as required. The above described method is

also termed "directed evolution" of the compound since it involves a multitude of steps including modification and selection, whereby binding compounds are selected in an "evolutionary" process optimizing their capabilities with respect to a particular property, e.g. its binding activity, its ability to activate, inhibit or modulate the activity, in particular inhibit the intracellular release of calcium mediated by the polypeptides of the present invention.

Of particular interest are compounds that antagonize the bitter taste receptor activity of the TAS2Rs disclosed and described herein. The specification thereby enables the skilled person to design intelligent compound libraries to screen for antagonists to the bitter response of these receptors, which in turn enables the development of compounds and compositions to suppress or eliminate bitter tasting components of foods, in particular animal foods, nutrients and dietary supplements and pharmaceutical or homeopathic preparations containing such phyto-chemicals. Similarly, the invention also enables the skilled person to screen for additional bitter ligands, or even to screen for compounds that enhance a bitter response, such as might be useful in the food industry. Therefore, another aspect of the invention is a process for isolating an antagonist of the bitter taste receptor activity of the polypeptide encoded by a polynucleotide selected from the group consisting of:

(a) polynucleotides encoding at least the mature form of the polypeptide having the deduced amino acid sequence as shown in SEQ ID NO to the at s 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48 and 50;
(b) polynucleotides having the coding sequence, as shown in SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47 and 49 encoding at least the mature form of the polypeptide;
(c) polynucleotides encoding a fragment or derivative of a polypeptide encoded by a polynucleotide of any one of (a) to (b), wherein in said derivative one or more amino acid residues are conservatively substituted compared to said polypeptide, and said fragment or derivative has bitter taste receptor activity;
(d) polynucleotides which are at least 50% identical to a polynucleotide as defined in any one of (a) to (c) and which code for a polypeptide having bitter taste receptor activity; and
(e) polynucleotides the complementary strand of which hybridizes, preferably under stringent conditions to a polynucleotide as defined in any one of (a) to (d) and which code for a polypeptide having bitter taste receptor activity;

comprising:

(1) contacting said polypeptide or a host cell genetically engineered with said polynucleotide or with a vector containing said polynucleotide with a potential antagonist;

(2) determining whether the potential antagonists antagonizes the bitter taste receptor activity of said polypeptide.

The polynucleotide employed in this process encodes a polypeptide that still exhibits essentially the same activity as the respective mature bitter taste receptor, i.e. has "bitter taste receptor activity". Preferably the polypeptide has at least 20% (e.g., at least: 20%; 30%; 40%; 50%; 60%; 70%; 80%; 90%; 95%; 98%; 99%; 99.5%; or 100% or even more) of the activity of the respective full-length TAS2R. One preferred way of measuring TAS2R activity is the ability to release intracellular calcium in a heterologous cell expression system like, for example, (HEK293/15) that stably expresses the alpha-subunit of promiscuous G-proteins, e.g. the mouse G_{15} subunit or chimeric , in response to bitter tastants, which is dependent on the expression of polypeptides encoded by the polynucleotides of the present invention. The amount of intracellular calcium released can be monitored by, for example, the *in vitro* FLIPR assay described herein but also by the measurement of one of a variety of other parameters including, for example, IP_3 or cAMP. Additional ways of measuring G-protein coupled receptor activity are known in the art and comprise without limitation electrophysiological methods, transcription assays, which measure, e.g. activation or repression of reporter genes which are coupled to regulatory sequences regulated via the respective G-protein coupled signaling pathway, such reporter proteins comprise, e.g., CAT or LUC; assays measuring internalization of the receptor; or assays in frog melanophore systems, in which pigment movement in melanophores is used as a read out for the activity of adenylate cyclase or phospholipase C (PLC), which in turn are coupled via G-proteins to exogenously expressed receptors (see, for example, McClintock T.S. et al. (1993) Anal. Biochem. 209: 298-305; McClintock T.S. and Lerner M.R. (1997) Brain Res. Brain, Res. Protoc. 2: 59-68, Potenza MN (1992) Pigment Cell Res. 5: 372-328, and Potenza M.N. (1992) Anal. Biochem. 206: 315-322)

As described above with the exception of hTAS2R40, single nucleotide polymorphisms are known for all of the above hTAS2Rs, which can be employed in a process for isolating an antagonist of the bitter taste receptor activity. Polynucleotides or polypeptides that differ from the respectively in SEQ ID 1-50 indicated sequences by the nucleotide and amino

acid change as indicted in Table I can similarly be employed for the process of the present invention.

The term "contacting" has the meaning as outlined above. A potential antagonist is a substance which lowers the respective bitter taste receptor activity determined in the absence of the antagonist by at least 10% (e.g., at least: 1%, 15% 20%; 30%; 40%; 50%; 60%; 70%; 80%; 90%; 95%; 98%; 99%; 99.5%; or 100%) once contacted with the bitter taste receptor.

In a preferred embodiment the process further comprises the contacting of the polypeptide with an agonist of the respective bitter taste receptor activity. The contacting of the bitter taste receptor with the agonist can be carried out prior, concomitantly or after contacting the polypeptide with the potential antagonist.

It has been demonstrated by the inventors that the bitter receptors hTAS2R10, hTAS2R14, hTAS2R16, hTAS2R38, hTAS2R43, hTAS2R44, hTAS2R45, hTAS2R46 and hTAS2R48 respond with specificity to (a) defined classe(s) of ligand(s) that include a class of useful phyto-chemicals in a functional expression assay. Therefore, in an even more preferred embodiment the polypeptides and agonist employed together in above process are selected from the group consisting of:

(a) the polypeptide encoded by the polynucleotide outlined above as determined by SEQ ID NO: 1 and SEQ ID NO: 2 and the agonist selected from the group consisting of acetylthiourea, N,N-dimethylthioformamide, N,N'-diphenylthiourea, N-ethylthiourea, 2-imidazolidinethione, 4(6)-methyl-2-thiouracil, N-methylthiourea, phenylthiocarbamid, 6-phenyl-2-thiouracil, 6-propyl-2-thiouracil, tetramethylthiourea, thioacetamide, thioacetanilide, 2-thiobarbituric acid, and 2-thiouracil and functional derivatives thereof;
(b) the polypeptide encoded by the polynucleotide of claim 1 or 2 as determined by SEQ ID NO: 9 and SEQ ID NO: 10 and the agonist selected from the group consisting of saccharin and functional derivatives thereof;
(c) the polypeptide encoded by the polynucleotide of claim 1 or 2 as determined by SEQ ID NO: 11 and SEQ ID NO: 12 and the agonist selected from the group consisting of saccharin and acesulfame K and functional derivatives thereof;
(d) the polypeptide encoded by the polynucleotide of claim 1 or 2 as determined by SEQ

ID NO: 13 and SEQ ID NO: 14 and the agonist selected from the group consisting of absinthine and functional derivatives thereof;

(e) the polypeptide encoded by the polynucleotide of claim 1 or 2 as determined by SEQ ID NO: 15 and SEQ ID NO: 16 and the agonist selected from the group consisting of absinthine and functional derivatives thereof;

(f) the polypeptide encoded by the polynucleotide of claim 1 or 2 as determined by SEQ ID NO: 19 and SEQ ID NO: 20 and the agonist selected from the group consisting of absinthine and functional derivatives thereof;

(g) the polypeptide encoded by the polynucleotide of claim 1 or 2 as determined by SEQ ID NO: 37 and SEQ ID NO: 38 and the agonist selected from the group consisting of strychnine, brucine, denatonium benzoate, and absinthine and functional derivatives thereof;

(h) the polypeptide encoded by the polynucleotide of claim 1 or 2 as determined by SEQ ID NO: 41 and SEQ ID NO: 42 and the agonist selected from the group consisting of tyrosine, preferably L-tyrosine, and other bitter tasting amino acids including, e.g., leucine, histidine phenylalanine and tryptophan, and functional derivatives thereof; and

(i) the polypeptide encoded by the polynucleotide of claim 1 or 2 as determined by SEQ ID NO: 43 and SEQ ID NO: 44 and the agonist selected from the group consisting of naphtyl-ß-D-glucoside, phenyl-ß-D-glucoside, salicin, helicin, arbutin, 2-nitrophenyl-ß-D-glucoside, 4-nitrophenyl-ß-D-glucoside, methyl-ß-D-glucoside, esculin, 4-nitrophenyl-ß-D-thioglucoside, 4-nitrophenyl-ß-D-mannoside, and amygdalin and functional derivatives thereof.

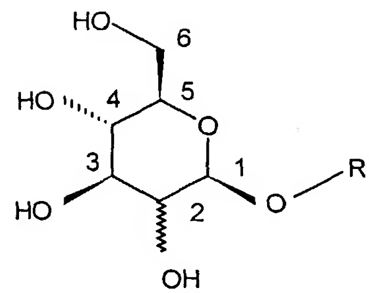
The term "functional derivatives thereof" refers to substances, which are derived from the respectively indicated bitter substance by chemical modification and which elicit at least 20% (e.g., at least: 20%; 30%; 40%; 50%; 60%; 70%; 80%; 90%; 95%; 98%; 99%; 99.5%; or 100% or even more) of the bitter taste receptor activity, if compared to the respective unmodified bitter substance. Chemical modification includes without limitation the introduction of one or more, preferably two, three or four novel side chains or residues or the exchange of one or more functional groups like, for example, introduction or exchange of H; linear or branched alkyl, in particular lower alkyl (C_1, C_2, C_3, C_4, and C_5, e.g. methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, tert-butyl, n-pentyl or iso-pentyl); substituted linear or branched alkyl, in particular lower substituted alkyl; linear or branched alkenyl, in

particular lower alkenyl (C_2, C_3, C_4 and C_5,, e.g. ethenyl, 1-propenyl, 2-propenyl, isopropenyl, 1-butenyl, 2-butenyl, 3-butenyl; substituted linear or branched alkenyl, in particular lower substituted alkenyl; linear or branched alkinyl, in particular lower alkinyl (C_2, C_3, C_4 and C_5); substituted linear or branched alkinyl, in particular lower substituted alkinyl; linear or branched alkanol, in particular lower alkanol (C_1, C_2, C_3, C_4, and C_5); linear or branched alkanal, in particular lower alkanal (C_1, C_2, C_3, C_4, and C_5, e.g. COH, CH_2COH, CH_2CH_2COH; aryl, in particular phenyl; substituted aryl, in particular substituted aryl; heteroaryl; substituted heteroaryl; alkylaryl, in particular benzyl; substituted alkylaryl; in particular substituted benzyl; alkylheteroaryl; substituted alkylheteroaryl; aminoalkyl, C_1, C_2, C_3, C_4 and C_5, e.g. $-NHCH_3$, $-NHCH_2CH3$, $-N(CH_3)_2$; substituted aminoalkyl; aminoketone, in particular $-NHCOCH_3$; substituted aminoketone; aminoaryl, in particular –NH-Ph; substituted aminoaryl, in particular substituted –NH-Ph; CN; NH_2; Halogen, in particular F, Cl, and Br; NO_2; OH; SH; NH; CN; or COOH group. If the residues mentioned above are substituted they are preferably mono, di, or tri substituted with a substituent selected from the group of halogen, in particular F, Cl, and Br, NH_2, NO_2, OH, SH, NH, CN, aryl, alkylaryl, heteroaryl, akylheteroaryl, COH or COOH.

In particular the hTAS2R16 receptor has been shown to respond specifically to a narrow class of interesting phyto-chemicals selected from the group consisting of bitter beta-glucopyranosides and mannopyranosides.

The beta-glucopyranosides and beta-mannosepyranosides are a group of bitter compounds consisting of a hydrophobic residue attached to glucose and mannose, respectively, by a beta-glycosidic bond.

Preferred compounds that bind to the hTAS2R16 taste receptor are chosen from beta glucopyranosides and beta- mannopyranosides defined by the formula:



These compounds were studied *in vitro* (see Table I and IV below) and also by human panelists (see Table I below) as is described in greater detail below.

From these studies certain inferences can be drawn regarding the affinity of the compounds towards activation of the hTAS2R16 receptor. Thus, for the promotion of activation the steric position at C2 can be either alpha or beta and the beta-configuration of the glycosidic bond and the alpha steric position of the hydroxyl group at C4 of the pyranose ring are preferred. Whereas R can be hydrogen, it is preferred that R is a substituent selected from C_1-C_8 alkyl which may be branched, linear or cyclic as appropriate; lower alkenyl residues, preferably having two, three, four or five carbon atoms; lower alkinyl residues, preferably having two, three, four or five carbon atoms, which can in a preferred embodiment be further substituted with F, Cl, Br, NH_2, NO_2, OH, SH, NH, CN, aryl, heteroaryl, COH or COOH group; heteroaryl, e.g. benzofuran and cumarin; aryl, e.g. phenyl, naphtyl; or the same of other sugar residue, e.g. glucopyranoside, which itself can carry a substituent R with the meaning as outlined above. Bulkier groups at C1 may increase the activation of the receptor. The aryl or heteroaryl may be further substituted with one or more substituents. Preferred substituents of the aryl or heteroaryl group are F, Br, Cl, NO_2, lower alkyl with one, two, three, four, five, six, seven or eight carbon atoms and CH_2OH. The phenyl group is preferably mono, di, or trisubstituted in ortho, para and/or meta position(s). The substituent at C6 is shown as an hydroxyl group above. However, the compounds activity as agonists are little effected by further or alternative substitution at this position, and there is design freedom at this part of the compound. Furthermore, without intending to be bound by theory, it is thought that the substituent "R" is not responsible for bitterness in these compounds. Rather, bitterness is thought to derive from a hydrogen acceptor and donor site provided by two hydroxyl groups on the ring. In another embodiment the O-glycosidic bond of the compounds outlined above can be a S-glycosidic bond, as exemplified by the bitter substance 4-nitrophenyl-ß-D-thioglucoside.

Most preferred compounds are selected from the group consisting of naphtyl-ß-D-glucoside, phenyl-ß-D-glucoside, salicin, helicin, arbutin, 2-nitrophenyl-ß-D-glucoside, 4-nitrophenyl-ß-D-glucoside, methyl-ß-D-glucoside, esculin, 4-nitrophenyl-ß-D-thioglucoside, 4-nitrophenyl-ß-D-mannoside, and amygdalin.

The beta-glucopyranosides are phytonutrients that represent an important class of compounds found in plant-derived foods that may be useful as dietary supplements, or in functional foods or medicaments for the prevention of disease states. However, due to their bitter after-taste they are aversive to consumers and so they are routinely removed from foods during production and processing as is further described in Drewnowski, A. & Gomez-Carneros, C. Bitter taste, phytonutrients, and the consumer: a review. *Am. J. Clin. Nutr.* **72**, 1424-1435 (2000). Removal is laborious and therefore expensive. The alternative is to mask the off-flavor using encapsulation technologies or organoleptic compounds as masking agents. However, encapsulation technology may not be appropriate in pharmaceutics as this may affect the absorption characteristics of the active compound, whereas the use of masking agents may impart their own characteristic flavor which may unbalance the flavor of food or beverages.

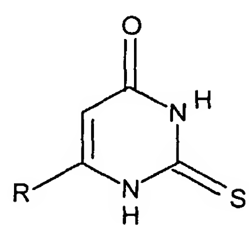
Without wishing to be bound by any particular theory as to their mechanism of action, applicant believes that the bitter receptors activate a G-protein and thereby initiate the aforementioned cellular activation cascade as a result of conformational changes in the receptor after binding by a ligand. Potential antagonists of the bitter response will contain functionality (i.e., will compete for binding at the receptor, and/or act at another binding site through an allosteric mechanism, and/or stabilize the receptor in the inactive conformation, and/or bind reversibly or irreversibly, and/or weaken receptor G protein interaction, and/or interfere with G protein activation).

Similarly, in another embodiment of the invention, it has been found that the so-called hTAS2R10 receptor is activated by strychnine, and strychnine analogues such as brucine as well as by denatonium benzoate, absinthine and other alkaloids with (a) ring system(s). Strychnine and its analogues are also useful phytochemicals that find use in medicines and homeopathic treatments.

In another embodiment of the invention, it has been found that the so-called hTAS2R14 receptor is activated by tyrosine, in particular L-tyrosine, and other bitter tasting amino acids including leucine, histidine, phenylalanine and tryptophan.

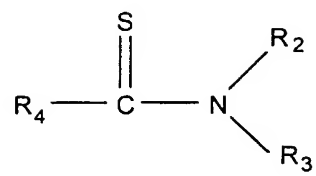
In another embodiment of the invention, it has been found that the so-called hTAS2R38 receptor is activated by acetylthiourea, N,N-dimethylthioformamide, N,N'-diphenylthiourea, N-ethylthiourea, 2-imidazolidinethione, 4(6)-methyl-2-thiouracil, N-methylthiourea, phenylthio-carbamid, 6-phenyl-2-thiouracil, 6-propyl-2-thiouracil, tetramethylthiourea, thioacetamide, thioacetanilide, 2-thiobarbituric acid, and 2-thiouracil.

From these studies certain inferences can be drawn regarding the affinity of the compounds, which activate the hTAS2R38 receptor. Thus, for the promotion of activation derivatives of 2-thiouracil according to following formula are preferred compounds.



Whereas R in this formula can be hydrogen, it is preferred that R is a substituent selected from C_1-C_{10} alkyl, which may be branched, linear or cyclic as appropriate, particularly preferred alkyls are methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, tert-butyl, n-pentyl or iso-pentyl residues; lower alkenyl residues, preferably having two, three, four or five carbon atoms; lower alkynyl residues, preferably having two, three, four or five carbon atoms, which can in a preferred embodiment be further substituted with F, Cl, Br, NH_2, NO_2, OH, SH, NH, CN, aryl, heteroaryl, COH or COOH group; heteroaryl, e.g. benzofuran and cumarin; aryl, e.g. phenyl, naphtyl; F, Cl, Br, NH_2, NO_2, OH, SH, NH, CN, aryl, alkylaryl, heteroaryl, alkylheteroaryl, COH or COOH group. In a further embodiment the carbon atom at the 4 position can substituted with -O-R_1 in which R_1 can have the same meaning as outlined above for R.

Another general structure of compounds having affinity for hTAS2R38 and which are thus suitable for activation of hTAS2R38 is depicted by the following formula:



In this formula R_2, R_3, and R_4 can each independently of each other have the meaning H; alkyl, in particular lower alkyl (C_1-C_5, e.g. methyl, ethyl, n-propyl, isopropyl, n-butyl, iso-butyl, tert-butyl, n-pentyl or iso-pentyl); substituted alkyl; alkenyl, in particular lower alkenyl (C_2-C_5); substituted alkenyl; alkinyl, in particular lower alkinyl (C_2-C_5); substituted alkinyl: alkanal, in particular lower alkanal (e.g. $-COCH_3$, $-COCH_2CH_3$, $-COCH_2CH_2CH_3$); aryl, in particular phenyl; substituted aryl; heteroaryl; substituted heteroaryl; alkylaryl, in particular benzyl; substituted alkylaryl; alkylheteroaryl; substituted alkylheteroaryl aminoalkyl, in particular $-NHCH_3$, $-NHCH_2CH_3$, $-N(CH_3)_2$; substituted aminoalkyl; aminoketone, in particular $-NHCOCH_3$; substituted aminoketone; aminoaryl, in particular –NH-Ph; substituted aminoaryl; CN; NH_2; Halogen, in particular F, Cl, and Br; NO_2. In a preferred embodiment R_2 or R_3 and R_4 can form a ring, preferably a four, five, six, seven or eight membered hetero cycle, which in a preferred embodiment is an aromatic hetero cycle. The residue of R_2 or R_3, which is not involved in the formation of the ring structure can have any of the meanings as outlined above. In a further preferred embodiment at least one of R_2 or R_3 has the meaning alkanal, preferably lower alkanal as outlined above. In case that only one of R_2 or R_3 has the meaning alkanal, than the other substituent preferably has the meaning H.

In a preferred embodiment R_2 is selected from the group consisting of H, CH_3 and Ph, R_3 is selected from the group of H, CH_3 and Ph and R_4 is selected from the group consisting of H, CH_3, NH-Ph, $-NHCH_2CH_3$, $-NHCH_2CH_3$, $-NHCH_3$, and $-N(CH_3)_2$.

In another embodiment of the invention, it has been found that the so-called hTAS2R43 receptor is activated by saccharin, derivatives thereof and other sulfoneimids.

In another embodiment of the invention, it has been found that the so-called hTAS2R44 receptor is activated by saccharin and acesulfame K, derivatives thereof and other sul-

foneimids.

In another embodiment of the invention, it has been found that the so-called hTAS2R45, hTAS2R46 and hTAS2R48 receptor is activated by absinthine derivatives thereof and other sulfoneimids.

The skilled person will appreciate that having regard to the structure-function information provided by the present invention, it is possible to compile libraries of molecules to find inhibitors of the bitter response of the disclosed hTAS2R in particular of the hTAS2R10, 14, 16, 38, 43, 44, 45, 46, and 48, which are triggered by the above outlined specific bitter substance(s). Such inhibitors, and libraries comprising same, form other aspects of the present invention. A still further aspect of the invention relates to the use of such inhibitors in food or pharmaceutical compositions containing bitter tastants such as referred to herein above, for the elimination or suppression of bitter taste perception.

In practicing the various aspects and embodiments of the present invention in relation to cloning receptors, elucidating ligand-receptor pairs, and finding modulators of the bitter response of receptors, recourse is made to conventional techniques in molecular biology, microbiology and recombinant technology. Accordingly, the skilled person is fully apprised of such techniques and as such they are hereafter treated only summarily in order to more fully describe the context of the present invention.

In order to express cDNAs encoding the receptors, one typically subclones receptor cDNA into an expression vector that contains a strong promoter to direct transcription, a transcription/translation terminator, and a ribosome-binding site for translational initiation. Suitable bacterial promoters are well known in the art, e.g., *E. coli*, *Bacillus sp.*, and *Salmonella*, and kits for such expression systems are commercially available. Similarly eukaryotic expression systems for mammalian cells, yeast, and insect cells are well known in the art and are also commercially available. The eukaryotic expression vector may be, for example an adenoviral vector, an adeno-associated vector, or a retroviral vector.

In addition to the promoter, the expression vector typically contains a transcription unit or expression cassette that contains all the additional elements required for the expression of the receptor-encoding nucleic acid in host cells. A typical expression cassette thus contains

a promoter operatively linked to the nucleic acid sequence encoding the receptor and signals required for efficient polyadenylation of the transcript, ribosome binding sites, and translation termination. The nucleic acid sequence encoding the receptor may typically be linked to a membrane-targeting signal such as the N-terminal 45 amino acids of the rat Somatostatin-3 receptor sequence to promote efficient cell-surface expression of the recombinant receptor. Additional elements of the cassette may include, for example enhancers.

An expression cassette should also contain a transcription termination region downstream of the structural gene to provide for efficient termination. The termination region may be obtained from the same gene as the promoter sequence or may be obtained from different genes.

The particular expression vector used to transport the genetic information into the cell is not particularly critical. Any of the conventional vectors used for expression in eukaryotic or prokaryotic cells may be used. Standard bacterial expression vectors include plasmids such as pBR322 based plasmids, pSKF, pET23D, and fusion expression systems such as GST and LacZ, but there are many more known in the art to the skilled person that can be usefully employed.

Expression vectors containing regulatory elements from eukaryotic viruses are typically used in eukaryotic expression vectors, e.g., SV40 vectors, papilloma virus vectors, and vectors derived from Epstein-Barr virus. Other exemplary eukaryotic vectors include pMSG, pAV009/A.sup.+, pMTO10/A.sup.+, pMAMneo-5, baculovirus pDSVE, pcDNA3.1, pIRES and any other vector allowing expression of proteins under the direction of the SV40 early promoter, SV40 late promoter, metallothionein promoter, murine mammary tumor virus promoter, Rous sarcoma virus promoter, polyhedrin promoter, or other promoters shown effective for expression in eukaryotic cells.

Some expression systems have markers that provide gene amplification such as thymidine kinase, hygromycin B phosphotransferase, and dihydrofolate reductase. Alternatively, high yield expression systems not involving gene amplification are also suitable.

The elements that are typically included in expression vectors also include a replicon that

functions in *E. coli*, a gene encoding drug resistance to permit selection of bacteria that harbor recombinant plasmids, and unique restriction sites in nonessential regions of the plasmid to allow insertion of eukaryotic sequences. The particular drug resistance gene chosen is not critical, any of the many drug resistance genes known in the art are suitable. The prokaryotic sequences are optionally chosen such that they do not interfere with the replication of the DNA in eukaryotic cells, if necessary.

Standard transfection methods can be used to produce bacterial, mammalian, yeast or insect cell lines that express large quantities of the receptor, which are then purified using standard techniques.

Any of the well known procedures for introducing foreign nucleotide sequences into host cells may be used. These include the use of calcium phosphate transfection, polybrene, protoplast fusion, electroporation, liposomes, microinjection, plasma vectors, viral vectors and any of the other well known methods for introducing cloned genomic DNA, cDNA, synthetic DNA or other foreign genetic material into a host cell. It is only necessary that the particular genetic engineering procedure used be capable of successfully introducing at least one gene into the host cell capable of expressing the receptor.

After the expression vector is introduced into the cells, the transfected cells may be cultured under conditions favoring expression of the receptor, which is recovered from the culture using standard techniques. For example the cells may be burst open either mechanically or by osmotic shock before being subject to precipitation and chromatography steps, the nature and sequence of which will depend on the particular recombinant material to be recovered. Alternatively, the recombinant protein may be recovered from the culture medium in which the recombinant cells had been cultured.

The activity of any of the receptors described herein can be assessed using a variety of *in vitro* and *in vivo* assays to determine functional, chemical, and physical effects, e.g., measuring ligand binding, secondary messengers (e.g., cAMP, cGMP, IP_3, DAG, or Ca^{2+}) ion flux, phosphorylation levels, transcription levels, neurotransmitter levels, and the like. Furthermore, such assays can be used to test for inhibitors of the receptors as is well known in the art.

Samples or assays that are treated with a potential receptor inhibitor may be compared to control samples without the test compound, to examine the extent of modulation. Control samples (untreated with inhibitors) are assigned a relative receptor activity value of 100. Inhibition of receptor activity is achieved when the receptor activity value relative to the control is lower, and conversely receptor activity is enhanced when activity relative to the control is higher.

The effects of the test compounds upon the function of the receptors can be measured by examining any of the parameters described above. Any suitable physiological change that affects receptor activity can be used to assess the influence of a test compound on the receptors of this invention. When the functional consequences are determined using intact cells or animals, one can measure a variety of effects such as changes in intracellular secondary messengers such as Ca^{2+}, IP_3 or cAMP.

Preferred assays for G-protein coupled receptors include cells that are loaded with ion sensitive dyes to report receptor activity. In assays for identifying modulatory compounds, changes in the level of ions in the cytoplasm or membrane voltage will be monitored using an ion sensitive or membrane voltage fluorescent indicator, respectively. For G-protein coupled receptors, promiscuous G-proteins such as G.alpha.15 and G.alpha.16 and chimeric G-proteins can be used in the assay of choice (see, for example, Wilkie et al., *Proc. Nat. Acad. Sci. USA* **88**, 10049-10053 (1991)). Such promiscuous G-proteins allow coupling of a wide range of receptors.

Receptor activation typically initiates subsequent intracellular events, e.g., increases in second messengers such as IP_3, which releases intracellular stores of calcium ions. Activation of some G-protein coupled receptors stimulates the formation of inositol triphosphate (1133) through phospholipase C-mediated hydrolysis of phosphatidylinositol (Berridge & Irvine, *Nature* **312**, 315-21 (1984)). IP_3 in turn stimulates the release of intracellular calcium ion stores. Thus, a change in cytoplasmic calcium ion levels, or a change in second messenger levels such as IP_3 can be used to assess G-protein coupled receptor function. Cells expressing such G-protein coupled receptors may exhibit increased cytoplasmic calcium levels as a result of contribution from both intracellular stores and via activation of ion channels, in which case it may be desirable, although not necessary, to conduct such assays in calcium-free buffer, optionally supplemented with a chelating agent such as

EGTA, to distinguish fluorescence response resulting from calcium release from internal stores.

In a preferred embodiment, receptor activity is measured by expressing the receptor in a heterologous cell with a promiscuous G-protein, such as G.alpha.15, 16, or a chimeric G-protein that links the receptor to a phospholipase C signal transduction pathway. Optionally the cell line is HEK-293, although other mammalian cells are also preferred such as CHO and COS cells. Modulation of taste transduction is assayed by measuring changes in intracellular Ca^{2+} levels, which change in response to modulation of the receptor signal transduction pathway via administration of a molecule that associates with the receptor. Changes in Ca^{2+} levels are optionally measured using fluorescent Ca^{2+} indicator dyes and fluorometric imaging.

The type of assay described above with respect to G-protein coupled bitter taste receptors can, however, also be employed for the identification of binding compounds, in particular agonists or antagonists of any G-protein coupled signalling molecule, in particular G-protein coupled receptor. Therefore, another aspect of the present invention relates to a process for the identification of agonists or antagonists of G-protein coupled signalling molecules comprising the steps of:

(1) contacting a cell comprising a promiscuous G-protein like, for example, G.alpha.15, 16, or a chimeric G-protein, and a G-protein coupled signalling molecule, in particular receptor, with a the potential agonist or antagonists of the signalling molecule;
(2) determining whether the potential agonist or antagonists agonizes or antagonizes the activity of the signalling molecule.

The activity of the signalling molecule and the increase or decrease of that activity in response to the potential agonist or antagonist can be determined as outlined above with respect to the identification of bitter receptor taste activity. The respectively indicated percent increases or decreases of the activity, which are required to qualify as antagonist or agonist do apply mutatis mutandis. Additionally the term "contacting" has the meaning as outlined above. Preferably the signalling molecule and/or the promiscuous G-protein has been introduced into the cell. The type of cell, which are preferred are those indicated above.

In yet another embodiment, the ligand-binding domains of the receptors can be employed *in vitro* in soluble or solid-state reactions to assay for ligand binding. Ligand binding in a receptor, or a domain of a receptor, can be tested in solution, in a bilayer membrane attached to a solid phase in a lipid monolayer or vesicles. Thereby, the binding of a modulator to the receptor, or domain, can be observed using changes in spectroscopic characteristics, e.g. fluorescence, absorbance or refractive index; or hydrodynamic (e.g. shape), chromatographic, or solubility properties, as is generally known in the art.

The compounds tested as modulators of the receptors can be any small chemical compound, or a biological entity, such as a protein, sugar, nucleic acid or lipid. Typically, test compounds will be small chemical molecules. Essentially any chemical compound can be used as a potential modulator or ligand in the assays of the invention, although knowledge of the ligand specificity of an individual receptor would enable the skilled person to make an intelligent selection of interesting compounds. The assays may be designed to screen large chemical libraries by automating the assay steps and providing compounds from any convenient source to assays, which are typically run in parallel (e.g., in microtiter formats on microtiter plates in robotic assays). The skilled person will understand that there are many suppliers of libraries of chemical compounds.

Assays may be run in high throughput screening methods that involve providing a combinatorial chemical or peptide library containing a large number of potential therapeutic, or tastant compounds (that are potential ligand compounds). Such libraries are then screened in one or more assays, as described herein, to identify those library members (particular chemical species or subclasses) that display a desired characteristic activity. The compounds thus identified can serve as lead compounds to further develop modulators for final products, or can themselves be used as actual modulators.

A combinatorial chemical library is a collection of diverse chemical compounds generated by either chemical synthesis or biological synthesis, by combining a number of chemical "building blocks" such as reagents. For example, a linear combinatorial chemical library such as a polypeptide library is formed by combining a set of chemical building blocks (amino acids) in every possible way for a given compound length (i.e., the number of amino acids in a polypeptide compound). Millions of chemical compounds can be synthesized through such combinatorial mixing of chemical building blocks.

Preparation and screening of combinatorial chemical libraries is well known to those of skill in the art and no more needs to be stated here.

In the high throughput assays of the invention, it is possible to screen up to several thousand different modulators or ligands in a single day. In particular, each well of a microtiter plate can be used to run a separate assay against a selected potential modulator, or, if concentration or incubation time effects are to be observed, every 5-10 wells can test a single modulator. Thus, a single standard microtiter plate can assay about 100 (e.g., 96) modulators. If 1536 well plates are used, then a single plate can easily assay from about 100 to about 1500 different compounds. It is possible to assay several different plates per day; assay screens for up to about 6,000-20,000 different compounds is possible using the integrated systems of the invention.

Lead compounds found by assay technology herein above described, or development compounds formed from such leads can be administered directly to a human subject to modulate bitter taste. Alternatively, such compounds can be formulated with other ingredients of preparations to be taken orally, for example, foods, including animal food, and beverages, pharmaceutical or nutraceutical or homeopathic preparations.

Therefore, another aspect of the invention is a process for the production of foodstuffs or any precursor material or additive employed in the production of foodstuffs comprising the steps of the above described processes for the identification of a compound binding to hTAS2R or an antagonist of hTAS2R and the subsequent step of admixing the identified compound or antagonist with foodstuffs or any precursor material or additive employed in the production of foodstuffs.

Bitter taste is a particular problem when orally administering pharmaceuticals, which often have an unpleasant bitter taste. In particular in elderly persons, children and chronically ill patients this taste can lead to a lack of compliance with a treatment regimen. In addition in veterinary applications the oral administration of bitter tasting pharmaceuticals can be problematic. Therefore, a further aspect of the invention is a process for the production of a nutraceutical or pharmaceutical composition comprising the steps of the processes of a compound binding to hTAS2R or an antagonist of hTAS2R and the subsequent step of

formulating the compound or antagonist with an active agent in a pharmaceutically acceptable form.

Consequently, a further aspect of the invention is a foodstuff, in particular animal food, or any precursor material or additive employed in the production of foodstuffs comprising an antagonist/inhibitor described above, preferably an antibody directed against one of the hTAS2Rs described herein, the extracellular domain of one of the hTAS2Rs described herein or an inhibiting RNA.

Also comprised is a nutraceutical or pharmaceutical composition comprising an antagonist/inhibitor as described above, preferably an antibody directed against one of the hTAS2Rs described herein, the extracellular domain of one of the hTAS2Rs described herein or an inhibiting RNA and an active agent, which preferably inhibits a bitter taste, and optionally a pharmaceutically acceptable carrier.

The amount of compound to be taken orally must be sufficient to effect a beneficial response in the human subject, and will be determined by the efficacy of the particular taste modulators and the existence, nature, and extent of any adverse side-effects that accompany the administration of a particular compound. There now follows a series of examples that serve to illustrate the invention, not to limit.

A further aspect of the present invention is the use of a polynucleotide as described above, a vector as described above, an antibody as described above or an antagonist/inhibitor of as described above, preferably an antibody directed against one of the hTAS2Rs described herein, the extracellular domain of one of the hTAS2Rs described herein or an inhibiting RNA for the manufacture of a medicament for the treatment of an abnormally increased or decreased sensitivity towards a bitter substance.

Techniques associated with detection or regulation of genes are well known to skilled artisans. Such techniques can be used, for example, for basic research on bitter receptors and to diagnose and/or treat disorders associated with aberrant bitter receptor expression.

The following examples are merely illustrative of the present invention and should not be construed to limit the scope of the invention as indicated by the appended claims in any

way. The contents of the US provisional application Ser. No. 60/413,298 the priority of which is claimed is hereby incorporated by reference in its entirety.

Example 1: Cloning of the hTAS2R genes.

Human genomic DNA was isolated from HEK293 cells using the E.Z.N.A. Blood DNA Kit II (Peqlab) and the various hTAS2Rs were amplified by PCR using gene-specific primers that span the complete coding region of the individual hTAS2R genes. Reaction parameters were: 4 cycles; 1 min, 94°C; 1 min, 64°C; 1.5 min 68°C using Advantage 2 polymerase (Clontech). 5% of the reaction served then as template for further amplification with Pfu DNA polymerase (Promega): 30 cycles; 1 min, 94 C; 1 min, 64°C; 3 min, 72°C. The hTAS2R amplicons were then sub-cloned into a cassette based on pcDNA5-FRT (Invitrogen). The cloning cassette contains the first 45 amino acids of the rat somatostatin type 3 receptor (as is further described by Meyerhof et al., *Proc. Nat. Acad. Sci. USA*, **89**, 10267-10271 (1992)) as a cell surface-targeting signal at the N-terminus. The C-terminus contained the herpes simplex virus (HSV) glycoprotein D epitope which does not interfere with signaling of heptahelical receptors and can be used for immunocytochemistry using an antibody that binds specifically to the HSV glycoprotein D epitope (see Roosterman et al, *J. Neuroendocrinol*, **9**, 741-751 (1997)). Comparison of the DNA sequences of at least four clones identified mutations generated during PCR and this avoided picking mutated clones. We compared the amino acid sequences using the AlignX program of the Vector NTI™ Suite (InforMax).

Using the above-described method, DNA sequences encoding all 24 bitter receptors identified by applicant were cloned. As indicated above, they were derived by a PCR-based method using genomic DNA as the template. Since all of the 24 genomic sequences lack introns, the DNA clones obtained had the same sequences as corresponding cDNA clones derived by reverse transcription-PCR (RT-PCR) of mRNA from cells expressing the relevant polypeptides would have.

Example 2: Immunocytochemistry

Batches of HEK293 cells were separately transiently transfected with expression vectors (pCDN5/FRT; Invitrogen) containing each of the 24 above described coding sequences

using lipofectamine 2000 (Invitrogen) and aliquots of the resulting cell populations were separately seeded on polylysine-coated coverslips. At 24 h post transfection they were washed with phosphate buffered saline (PBS), cooled on ice and added 20 microgram / ml biotin-labeled concanavalin A (Sigma) for 1 h, which binds to cell surface glycoproteins. Thereafter, the cells were fixed for 5 min in methanol/acetone (1:1) and then permeabilized for 4 min with 0.25% Triton X-100. In order to reduce nonspecific binding the coverslips were incubated in 2% goat serum. Thereafter, anti-HSV glycoprotein D antiserum (Novagen, 1:10,000) was added to detect the chimeric receptors that, as described above, would have a HSV glycoprotein epitope fused to their C-temini, and Texas Red-Avidin D (Vector, 1:200) has added to stain the cell surface and incubation continued overnight at 4 °C. Such C-termini are intracellular and for this reason it is necessary to permeabilize the cells to permit entry of the HSV glycoprotein D epitope-specific antibody molecules into them. After washing (5x in PBS, RT) Alexa488-conjugated goat anti-mouse antiserum (Molecular Probes, 1:1000) was added and incubation continued at room temperature for 1 h. Finally, the cells were embedded in Fluorescent Mounting Medium (Dako) and analyzed using a Leica TCS SP2 Laser Scan Inverted microscope. The preparations were scanned sequentially with an argon/krypton laser (488 nm) to excite the Alexa488 dye and with a green-helium-neon laser (543 nm) to excite the Texas Red dye. The spectral detector recorded light emission at 510-560 nm and 580-660 nm, respectively. Images of 1024 X 1024 pixels were processed with Corel PHOTO-PAINT 10.0 (Corel Corporation) and printed on a Tektronix color laser printer. The immunocytochemical data permitted calculation of the proportion of cells expressing recombinant receptors (green fluorescent cells divided by total cell number in a microscopic field) and the proportion of cells that display expression of TAS2Rs at the plasma membrane level (number of cells with colocalization of green and red fluorescence divided by the number of green fluorescent cells). Of the 24 transfectant lines tested, all were found to express the encoded polypeptides. The proportion of receptor-expressing cells in the various transfectant lines ranged from about 10% to about 35%.

Example 3: Heterologous Expression of hTAS2R Receptors

A fluorescence imaging plate reader (FLIPR, Molecular Devices) was used to functionally screen cell populations transiently transfected with expression vectors encoding the above-described 24 bitter receptors and to establish concentration-response curves for hTAS2R16

and hTASR10. The single-cell calcium imaging technique was also employed to demonstrate receptor selectivity and crossdesensitization. For the FLIPR experiments the HEK293/15 cells were grown to 50% confluence. The cells were then seeded at a density of 3 x 10^3 cells per well into 96-well black-wall, clear-bottom microtiter plates (Greiner). After 48 h the cells in each well were transfected using Lipofectamine 2000 and 24-30 h later were loaded with Fluo4AM (Molecular Probes). Thereafter they were stimulated with bitter compounds (SigmaAldrich, further purified by reversed-phase HPLC to > 99% purity). Calcium signals were recorded simultaneously from each well at 1 Hz at 510 nm after excitation at 488 nm and the recordings were corrected for cell density. The responses of five wells containing cells expressing the same receptor and that received the same stimulus (i.e., the same compound at the same concentration) were averaged. Calcium traces were subtracted that were determined in triplicate of mock-transfected cells stimulated with the same concentration of tastant. The calculations rest on at least four independent transfection experiments. Plots of the amplitudes versus concentrations fitted by nonlinear regression to the function $f(x)=100/(1+(EC_{50}/x)^{nH})$, with x = agonist concentration and nH = Hill coefficient permitted calculation of EC_{50} values and threshold values of activation.

EC_{50} and threshold values obtained with hTAS2R16-expressing transfectants are shown in Table 1 below and the results are described in Example 4.

In separate experiments, hTAS2R10-expressing transfectants were found to have a threshold of activation of approximately 0.1 μM and a EC_{50} of 5-20 μM using strychnine as the test compound. Similar results were obtained with brucine.

Single-cell Ca^{2+} imaging was performed with the hTAS2R16-transfected HEK293/15 cells as described in *Cell* **95**, 917-926 (1998), but with the following modifications: The Till Photonics imaging system (Munich, Germany) was used in which a monochromator is connected by a quartz fiber lightguide and an epifluorescence condenser to an inverted Olympus IX50 microscope equipped with a UApo/340 40x1.35 oil-immersion lens. 30 h post-transfection, FURA-2AM-loaded cells were sequentially illuminated in 5 s intervals for 3-10 ms, first at 340 nm, then at 380 nm, online ratioed light emissions at 510 nm (340/380) and monitored the images via an intensified, cooled CCD camera. The 5 s interval camera pictures of all cells in the microscope field of vision permanently were stored and analyzed offline. 10-15% of all cells in the camera field responded to agonists in tran-

sient transfection experiments. The proportion of responders was about half of that found by immunocytochemistry, probably reflecting a sub-optimal signal transduction. Responses were not observed in mock-transfected cells. Isoproterenol (10 microMolar) was used at the end of all experiments to stimulate endogenous betaadrenergic receptors, proving a functional $G_{alpha\ 15}$ dependent signal transduction cascade.

For RT-PCR and *in-situ* hybridization work, human RNA (Clontech) was purchased or it was isolated from surgical tongue specimens with peqGOLD RNAPure (Peqlab) and the preparations digested with DNase I (Invitrogen). Following cDNA synthesis (Smart cDNA synthesis Kit, Clontech) hTAS2R16 cDNA was PCR-amplified (39 cycles, 1 min 94°C, 1 min 64°C, 1 min 72°C) using specific forward and reverse primers with overhangs containing EcoRI or NotI sites SEQ ID Nos 51 and 52 and the amplicons analyzed on agarose gels. Subcloning and sequencing demonstrated the identity of the amplified bands. Approximately 15 micrometer cryo-sections of human tongue specimens containing vallate papilla at 65°C were processed and hybridized with a hTAS2R16 riboprobe spanning the complete coding region and generated from hTAS2R16 cDNA. The *in-situ* hybridization method used was essentially the same as that described in *Nature*, **413**, 631-635 (2001) except that the riboprobe was conjugated with biotin and an alkaline phosphatase-avidin conjugate was used for detection. This experiment indicated that TAS2R16 mRNA is expressed in vallate papilla which are known to perceive bitter taste.

<u>Example 4: Human Taste Experiments</u>

15 experienced panelists in a sensory panel room at 22-25 °C determined bitter thresholds on three different sessions using a triangle test with tap water as solvent, according to methodology set out in *J. Agric. Food Chem.*, **49**, 231-238 (2001), or Mailgaard M et al, "*Sensory Evaluation Techniques*" (CRC Press LLC, New York 1999). For dose-response relations, bitter tastant concentration series were presented to 10 trained panelists in random order. The panelists ranked the samples in increasing order of intensity and, for each concentration, evaluated bitterness intensity on a scale from 0 to 5 (ref. 24). The dose-response curves of three different sessions were averaged. The intensity values between individuals and separate sessions differed by not more than 0.5 units.

To investigate adaptation, the 8 panelists first maintained aqueous solutions (5 ml) of

phenyl-β-D-glucopyranoside (8 mM), phenyl-alpha-D-glucopyranoside (180 mM), salicin (8 mM), or helicin (8 mM) for 15 s in their oral cavities and evaluated the bitter intensity as described above. After 30 min, they kept a denatonium benzoate solution (5 ml, 0.0003 mM) for 15 s in their mouth and evaluated its bitterness. The panelists spat off the denatonium benzoate solution, took up the phenyl-β-D-glucopyranoside or the phenyl-alpha-D-glucopyranoside solutions orally for 120 s or 180 s and judged their bitterness intensity after 15, 30, 60, 120 and 180 s. Thereafter, the panelists spat off these solutions and then sequentially took up salicin, helicin (5 ml, 8 mM) and denatonium benzoate (5 ml, 0.0003 mM) and evaluated bitterness intensities of these solutions after 15 s. After an additional 30 min, the first experiment was repeated. The data of three different sessions for each panelist were averaged. Intensity values between individuals and separate sessions differed by not more than ±0.5 units.

Results of in vitro assays (FLIPR) and human taste experiments are shown in Table 1 below.

Table II

Compound	Threshold Value (mM)		EC_{50} (mM)	
	FLIPR	Human	FLIPR	Human
1	0.07+/- 0.02	0.1 +/- 0.05	1.1 +/- 0.1	0.7+/- 0.2
2	0.07+/- 0.02	0.2+/-0.1	1.4+/- 0.2	1.1 +/-0.3
3	0.3+/- 0.1	0.4+/- 0.1	2.3+/- 0.4	2.2+/- 0.7
4	0.5+/- 0.2	0.9+/- 0.3	5.8+/- 0.9	5.4+/-1.8
5	1.5+/- 0.5	n.d.	n.d.	n.d.
6	0.4+/-0.1	0.2+/-0.1	1.0+/-0.1	1.4+/-0.4
7	15+/-6	32+/-11	n.d.	320+/-108
8	2.3+/-0.9	n.d.	20+/-3.4	n.d.
9	4+/-2	4+/-1	n.d.	n.d.

10	n.r.	40+/-13	n.r.	n.d.
11	n.r.	9+/-3	n.r.	50+/-17

1 = phenyl-beta-D-glucopyranoside; 2= salicin; 3= helicin; 4= arbutin; 5= 2-nitro-phenyl-beta-D-glucopyranoside; 6= naphthyl-beta-D-pyranoside; 7= methyl-beta-D-lucopyranoside; 8= amygdalin; 9= esculin; 10= phenyl-beta-D-galactopyranoside; 11= phenyl-alpha-D-glucopyranoside.

n.d. = not data due to solubility problems or toxicity or artifacts *in vitro*.

n.r. = No response up to 100 mM.

The FLIPR results provide the threshold concentration of the compounds (nM) at which point the receptor detects the compounds. The EC_{50} results express the concentration of the compound wherein the receptor signal is at 50%, and is a representation of the affinity of a receptor for a compound.

The results show that the *in vitro* FLIPR measurements for salicin closely resemble the human taste study results. This bitter-tasting compound has known anti-pyretic and analgesic action, and the results suggest that *in vitro* assays using hTAS2R16 may represent a useful tool to find compounds that suppress or eliminate the bitter response to this compound. Also, for all the other tested beta-glucopyranosides, the close correspondence of Threshold Concentration and EC_{50} results suggest that hTAS2R16 is a cognate human receptor for these class of bitter compounds. In contrast, the related structures (see compounds 10 and 11) show 90- to 400-fold higher Threshold Concentrations, which indicates that this receptor is rather selective, and that these bitter compounds activate different receptors.

Adaptation frequently occurs in sensory systems and means that stimuli elicit reduced responses upon prolonged or repeated stimulus presentations. Repeated stimulation of hTAS2R16-expressing cells with phenyl-beta-D-glucopyranoside resulted in largely diminished responses to salicin as well. This cross-desensitization occurred among the other tested beta-pyranosides and was fully reversible. It resembles homologous desensitization of agonist-occupied heptahelical receptors mediated by GRKs, i.e. specific kinases, and arrestins. We also observed adaptation in the human test panel that initially scored phenyl-beta-D-glucopyranoside, salicin and helicin as equally intensely bitter. The bitterness of

phenyl-beta-D glucopyranoside declined during prolonged stimulation and the test panel perceived salicin and helicin also as less bitter, but not the unrelated bitter substance denatonium benzoate, which cannot activate TAS2R16. Adaptation was fully reversible. On the opposite, the phenyl-alpha-D-glucopyranoside failed to cross-adapt with all tested beta-D-glucopyranosides, although its own bitter response desensitized strongly. This indicates that beta-glucopyranosides signal through a common mechanism most likely involving hTAS2R16 as a bitter taste receptor while the alpha-isomer activates a separate receptor. A recent human psychophysical study also revealed cross-adaptation amongst two bitter amino acids but not between the two bitter amino acids and urea, suggesting the existence of distinct receptors for the bitter amino acids and urea. Although most, if not all, bitter receptors are present in the same subset of taste receptor cells, adaptation to specific bitter stimuli can be explained if bitter receptors were subject to homologous desensitization.

Example 5 Heterologous Expression of hTAS2R in

Transient transfection of TAS2Rs into HEK-293T-Gα16gustducin44 cells. We cloned the DNAs of all human putative bitter responsive receptors into pCDNA5/FRT (Invitrogen) by PCR-methods and transiently transfected the plasmids with lipofectamine 2000 (Invitrogen) into HEK-293T-Gα16gustducin44 cells grown to 50% confluence. These cells stably express a chimeric G protein constructed from human Gα16 and rat gustducin. Finally, we seeded the transfected cells at a density of 3 x 10^3 cells per well into 96-well black-wall, clear-bottom microtiter plates (Greiner).

Co-transfection of TAS2Rs with gustducin and phospholipase-Cβ2 into HEK-293 cells. Alternatively, we transfected simultaneously plasmid DNAs encoding one of the TAS2Rs, phospholipase-Cβ2 and α-gustducin into HEK-293 cells using the lipofectamine method. Additional cotransfection of G-protein β and γ-subunits may improve the bitter tastant-induced responses. Thereafter, the transfected cells were seeded at a density of 3 x 10^3 cells per well into 96-well black-wall, clear-bottom microtiter plates (Greiner).

Fluorometric Imaging Plate Reader (FLIPR) assay 24-30 h later, the cells were loaded with 4 μM FLUO-4/AM (Molecular Probes) and 0.04% Pluronic F-127 (Molecular Probes) in Hepes-buffered saline (HBS), 140 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, 10 mM Hepes, 10 mM glucose and 2.5 mM probenicide, pH 7.4, for 1 hour at 37°C. Thereafter, cells were

gently washed in HBS by an automated plate washer (Denley Cellwash, Labsystems) and transferred to the FLIPR (Molecular Devices). The FLIPR integrates an argon laser excitation source, a 96-well pipettor, and a detection system utilizing a Charged Coupled Device imaging camera. Fluorescence emissions from the 96 wells were monitored at an emission wavelength of 510 nm, after excitation with 488 nm (F488). Fluorescence data were collected 1 min before and 10 min after stimulation. Data were collected every 6 s before and every 1 s after agonist stimulation. 50 µl of 3x concentrated agonists were delivered within 2 s by the integrated 96-well pipettor to the wells containing 100 µl HBS. Agonist responses were quantified using the amplitudes of the fluorescence peaks. We averaged the responses of five wells containing cells expressing the same receptor and that received the same stimulus. Calcium traces were determined in triplicate of mock-transfected cells stimulated with the same concentration of tastant. EC_{50} values and plots of the amplitudes versus concentrations were derived from fitting the data by nonlinear regression to the function $f(x) = 100 / [1 + (EC_{50}/x)^{nH}]$, where x is the agonist concentration and nH is the Hill coefficient. The results for hTAS2R10 (Table II), hTAS2R14 (Table III), hTAS2R16 (Table IV), hTAS2R38 (Table V), hTAS2R43 (Table VI), hTAS2R44 (Table VII), hTAS2R45 (Table VIII), hTAS2R46 (Table IX) and hTAS2R (Table X) are shown below.

Table III
Identified agonists of hTAS2R10

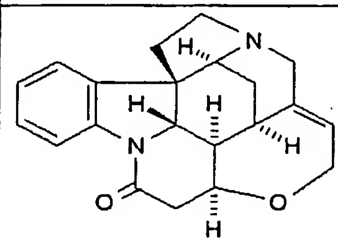
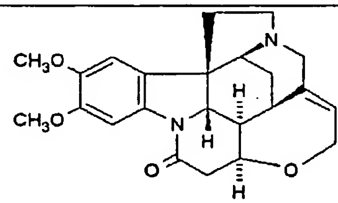
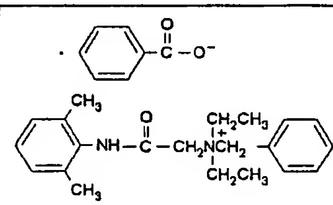
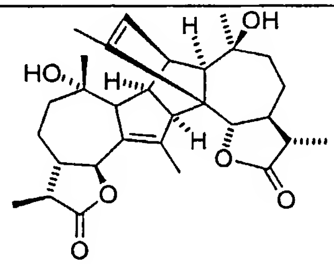
Substance	Structure	Approx. threshold [mM]	EC_{50} [mM]
Strychnine *		0.003	0.04
Brucine		0.01	0.06
Denatonium benzoate		0.003	0.07
Absinthine		0.01	

Table IV

Identified agonists of hTAS2R14

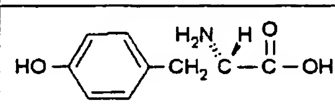
Substance	Structure	Reacts at
L-Tyrosine		1 mM

Table V
Identified agonists of hTAS2R16

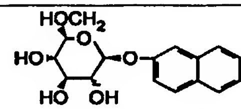
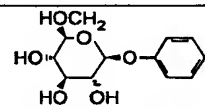
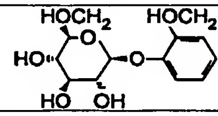
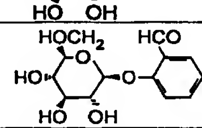
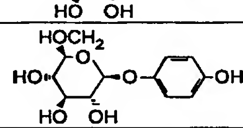
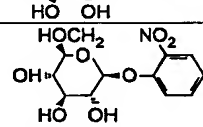
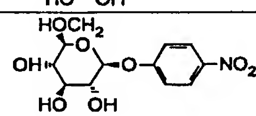
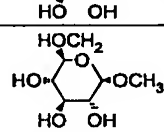
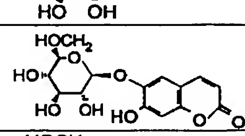
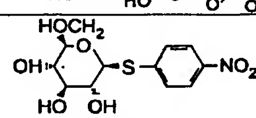
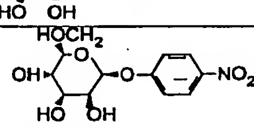
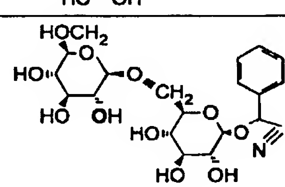
Substance	Structure	Threshold [mM]	EC_{50} [mM]
Naphtyl-ß-D-Glucoside		0.4 ± 0.1	1.0 ± 0.1
Phenyl –ß-D-Glucoside		0.07 ± 0.02	1,1 ± 0.1
Salicin		0.07 ± 0.02	1.4 ± 0.2
Helicin		0.3 ± 0.1	2.3 ± 0.4
Arbutin		0.5 ± 0.2	5.8 ± 0.9
2-Nitrophenyl-ß-D-Glucoside		0.3 - 1	Not determined
4-Nitrophenyl-ß-D-Glucoside		1 - 3	Not determined
Methyl-ß-D-Glucoside *		15 ± 6	32 ± 11
Esculin		4 ± 2	Not determined
4-Nitrophenyl-ß-D-Thioglucoside		1 - 5	Not determined
4-Nitrophenyl-ß-D-Mannoside		1 - 3	Not determined
Amygdalin		2.3 ± 0.9	20 ± 3.4

Table VI
Identified agonists of hTAS2R38

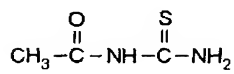
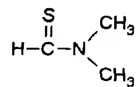
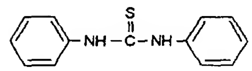
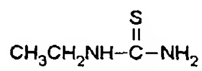
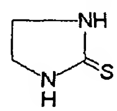
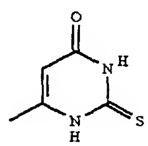
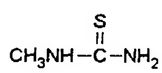
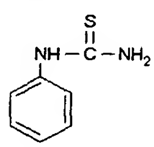
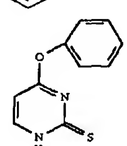
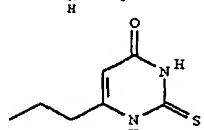
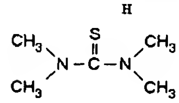
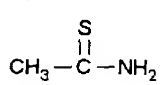
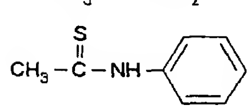
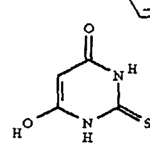
Substance	Structure	Approx. Threshold [μM]	EC_{50} [μM]
Acetylthiourea		2	15
N,N-Dimethyl-thioformamide		10	55
N,N'-Diphenylthiourea		0.3	2.3
N-Ethylthiourea		30	260
2-Imidazolidinethione (=N,N′-Ethylenethiourea)		10	not determined
4(6)-Methyl-2-thiouracil		20	180
N-Methylthiourea		100	estimated 600-800
Phenylthiocarbamid (PTC)		0.3	2
6-Phenyl-2-thiouracil		0.15	0.5
6-Propyl-2-thiouracil (PROP)		0.3	2
Tetramethylthiourea		10-30	100
Thioacetamide		100	not determined
Thioacetanilide		3	18
2-Thiobarbituric acid		reacts at 10 mM	

Table VI (continued)

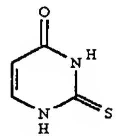
2-Thiouracil		300	estimated 2000

Table VII
Identified agonists of hTAS2R43

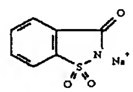
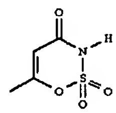
Substance	Structure	Approx. Threshold [mM]	EC_{50} [mM]
Saccharin		0.2	1.1
Acesulfame K		No response up to 10 mM	

Table VIII
Identified agonists of hTAS2R44

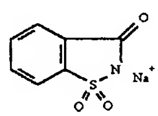
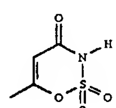
Substance	Structure	Approx. Threshold [mM]	EC_{50} [mM]
Saccharin		0.2	estimated 2-5
Acesulfame K		0.5	3

Table IX
Identified agonists of hTAS2R45

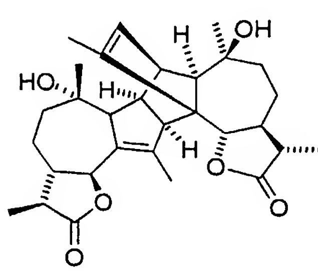
Substance	Structure	Approx. Threshold [mM]	EC_{50} [mM]
Absinthine		0.003	Not determined

Table X
Identified agonists of hTAS2R46

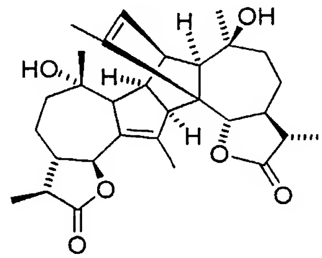
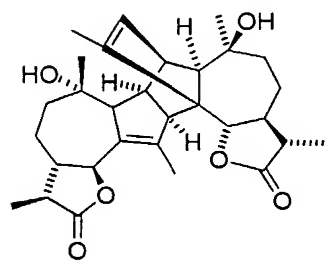
Substance	Structure	Approx. Threshold [mM]	EC_{50} [mM]
Absinthine		0.001	Not determined

Table XI
Identified agonists of hTAS2R48

Substance	Structure	Approx. Threshold [mM]	EC_{50} [mM]
Absinthine		0.03	Not determined

Claims

1. A polynucleotide selected from the group consisting of:
   (a) polynucleotides encoding at least the mature form of the polypeptide having the deduced amino acid sequence as shown in SEQ ID NOs 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24;
   (b) polynucleotides having the coding sequence, as shown in SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, and 23 encoding at least the mature form of the polypeptide;
   (c) polynucleotides encoding a fragment or derivative of a polypeptide encoded by a polynucleotide of any one of (a) to (b), wherein in said derivative one or more amino acid residues are conservatively substituted compared to said polypeptide, and said fragment or derivative has bitter substance binding activity;
   (d) polynucleotides which are at least 50% identical to a polynucleotide as defined in any one of (a) to (c) and which code for a polypeptide having bitter substance binding activity; and
   (e) polynucleotides the complementary strand of which hybridizes, preferably under stringent conditions to a polynucleotide as defined in any one of (a) to (d) and which code for a polypeptide having bitter substance binding activity;

   or the complementary strand of such a polynucleotide.

2. The polynucleotide of claim 1 which is DNA, genomic DNA or RNA.

3. A vector containing the polynucleotide of claim 1 or 2.

4. The vector of claim 3 in which the polynucleotide is operatively linked to expression control sequences allowing expression in prokaryotic and/or eukaryotic host cells.

5. A host cell genetically engineered with the polynucleotide of claim 1 or 2 or the vector of claim 3 or 4.

6. A transgenic non-human animal containing a polynucleotide of claim 1 or 2, a vector of claim 3 or 4 and/or a host cell of claim 5.

7. A process for producing a polypeptide encoded by the polynucleotide of claim 1 or 2 comprising: culturing the host cell of claim 5 and recovering the polypeptide encoded by said polynucleotide.

8. A process for producing cells capable of expressing at least one of the bitter taste receptor polypeptides comprising genetically engineering cells *in vitro* with the vector of claim 3 or 4, wherein said bitter taste receptor polypeptide(s) is(are) encoded by a polynucleotide of claim 1 or 2.

9. A polypeptide having the amino acid sequence encoded by a polynucleotide of claim 1 or 2 or obtainable by the process of claim 7.

10. An antibody specifically binding to the polypeptide of claim 9.

11. A nucleic acid molecule which specifically hybridizes to a polynucleotide of claim 1 or 2.

12. An antagonist/inhibitor against the polypeptide of claim 8 which is an antibody, the extracellular domain of the polypeptide of claim 8 or a fragment thereof or an inhibiting RNA.

13. The antagonist/inhibitor of claim 12, wherein said inhibiting RNA is an antisense construct hybridizing to a polynucleotide of claim 1 or 2, RNAi, siRNA or a ribozyme.

14. A process for isolating a compound that binds to a polypeptide encoded by a polynucleotide selected from the group consisting of:
   (a) polynucleotides encoding at least the mature form of the polypeptide having the deduced amino acid sequence as shown in SEQ ID NOs 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48 and 50;
   (b) polynucleotides having the coding sequence, as shown in SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47 and 49 encoding at least the mature form of the polypeptide;
   (c) polynucleotides encoding a fragment or derivative of a polypeptide encoded by a polynucleotide of any one of (a) to (b), wherein in said derivative one or more

amino acid residues are conservatively substituted compared to said polypeptide, and said fragment or derivative has bitter substance binding activity;

(d) polynucleotides which are at least 50% identical to a polynucleotide as defined in any one of (a) to (c) and which code for a polypeptide having bitter substance binding activity; and

(e) polynucleotides the complementary strand of which hybridizes, preferably under stringent conditions to a polynucleotide as defined in any one of (a) to (d) and which code for a polypeptide having bitter substance binding activity;

comprising:

(1) contacting said polypeptide or a host cell genetically engineered with said polynucleotide or with a vector containing said polynucleotide with a compound;

(2) detecting the presence of the compound which binds to said polypeptide; and

(3) determining whether the compound binds said polypeptide.

15. A process for isolating an antagonist of the bitter taste receptor activity of the polypeptide encoded by a polynucleotide selected from the group consisting of:

(a) polynucleotides encoding at least the mature form of the polypeptide having the deduced amino acid sequence as shown in SEQ ID NO to the at s 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48 and 50;

(b) polynucleotides having the coding sequence, as shown in SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47 and 49 encoding at least the mature form of the polypeptide;

(c) polynucleotides encoding a fragment or derivative of a polypeptide encoded by a polynucleotide of any one of (a) to (b), wherein in said derivative one or more amino acid residues are conservatively substituted compared to said polypeptide, and said fragment or derivative has bitter taste receptor activity;

(d) polynucleotides which are at least 50% identical to a polynucleotide as defined in any one of (a) to (c) and which code for a polypeptide having bitter taste receptor activity; and

(e) polynucleotides the complementary strand of which hybridizes, preferably under stringent conditions to a polynucleotide as defined in any one of (a) to (d) and which code for a polypeptide having bitter taste receptor activity;

comprising:

(1) contacting said polypeptide or a host cell genetically engineered with said

polynucleotide or with a vector containing said polynucleotide with a potential antagonist;

(2) determining whether the potential antagonists antagonizes the bitter taste receptor activity of said polypeptide.

16. The process of claim 15 further comprising the contacting of the polypeptide with an agonist of the respective bitter taste receptor activity.

17. The process of claim 16 in which said contacting with an agonist is carried out prior, concomitantly or after step (1) of claim 15.

18. The process of claim 16 or 17 in which said polypeptide and said agonist are selected from the group consisting of:

(a) the polypeptide encoded by the polynucleotide of claim 1 or 2 as determined by SEQ ID NO: 1 and SEQ ID NO: 2 and the agonist selected from the group consisting of acetylthiourea, N,N-dimethylthioformamide, N,N'-diphenylthiourea, N-ethylthiourea, 2-imidazolidinethione, 4(6)-methyl-2-thiouracil, N-methylthiourea, phenylthiocarbamid, 6-phenyl-2-thiouracil, 6-propyl-2-thiouracil, tetramethylthiourea, thioacetamide, thioacetanilide, 2-thiobarbituric acid, and 2-thiouracil and functional derivatives thereof;

(b) the polypeptide encoded by the polynucleotide of claim 1 or 2 as determined by SEQ ID NO: 9 and SEQ ID NO: 10 and the agonist selected from the group consisting of saccharin and acesulfame K and functional derivatives thereof;

(c) the polypeptide encoded by the polynucleotide of claim 1 or 2 as determined by SEQ ID NO: 11 and SEQ ID NO: 12 and the agonist selected from the group consisting of saccharin and acesulfame K and functional derivatives thereof;

(d) the polypeptide encoded by the polynucleotide of claim 1 or 2 as determined by SEQ ID NO: 13 and SEQ ID NO: 14 and the agonist selected from the group consisting of absinthine and functional derivatives thereof;

(e) the polypeptide encoded by the polynucleotide of claim 1 or 2 as determined by SEQ ID NO: 15 and SEQ ID NO: 16 and the agonist selected from the group consisting of absinthine and functional derivatives thereof;

(f) the polypeptide encoded by the polynucleotide of claim 1 or 2 as determined by SEQ ID NO: 19 and SEQ ID NO: 20 and the agonist selected from the group

consisting of absinthine and functional derivatives thereof;

(g) the polypeptide encoded by the polynucleotide of claim 1 or 2 as determined by SEQ ID NO: 37 and SEQ ID NO: 38 and the agonist selected from the group consisting of strychnine, brucine, denatonium benzoate, and absinthine and functional derivatives thereof;

(h) the polypeptide encoded by the polynucleotide of claim 1 or 2 as determined by SEQ ID NO: 41 and SEQ ID NO: 42 and the agonist selected from the group consisting of tyrosine, in particular L-tyrosine, leucine, phenylalanine, histidine, tryptophan and functional derivatives thereof; and

(i) the polypeptide encoded by the polynucleotide of claim 1 or 2 as determined by SEQ ID NO: 43 and SEQ ID NO: 44 and the agonist selected from the group consisting of naphtyl-ß-D-glucoside, phenyl-ß-D-glucoside, salicin, helicin, arbutin, 2-nitrophenyl-ß-D-glucoside, 4-nitrophenyl-ß-D-glucoside, methyl-ß-D-glucoside, esculin, 4-nitrophenyl-ß-D-thioglucoside, 4-nitrophenyl-ß-D-mannoside, and amygdalin and functional derivatives thereof.

19. A process for the production of a food or any precursor material or additive employed in the production of foodstuffs comprising the steps of the processes of any of claims 14 to 18 and the subsequent step of admixing the identified compound or antagonist with foodstuffs or any precursor material or additive employed in the production of foodstuffs.

20. A process for the production of a nutraceutical or pharmaceutical composition comprising the steps of the processes of any of claims 14 to 18 and the subsequent step of formulating the compound or antagonist with an active agent in a pharmaceutically acceptable form.

21. A food stuff, including human and animal food stuff, any precursor material or additive employed in the production of foodstuff comprising an antagonist/inhibitor of claim 12 or 13.

22. A nutraceutical or pharmaceutical composition comprising an antagonist/inhibitor of claim 12 or 13 and an active agent and optionally a pharmaceutically acceptable carrier.

23. Use of a polynucleotide of claim 1 or 2, a vector of claim 3 or 4, an antibody of claim 10 or an antagonist/inhibitor of claim 12 or 13 for the manufacture of a medicament for the treatment of an abnormally increased or decreased sensitivity towards a bitter substance.

SEQUENCE LISTING

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<211> 921
<212> DNA
<213> Homo sapiens

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gttgggacag tgcacaactt ctactactct gcccagaagg tcgagtactc tgggggtctc 240

ggccgacagt tcttccatct acactggcac ttcctgaact cagccacctt ctggttttgc 300

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tggctgaagt ggaggttcct agggtgggtg ccctggctcc tgttgggctc tgtcctgatc 420

tccttcatca taaccctgct gtttttttgg gtgaactacc ctgtatatca agaattttta 480

attagaaaat tttctgggaa catgacctac aagtggaata caaggataga aacatactat 540

ttcccatccc tgaaactggt catctggtca attccttttt ctgtttttct ggtctcaatt 600

atgctgttaa ttaattctct gaggaggcat actcagagaa tgcagcacaa cgggcacagc 660

ctgcaggacc ccagcaccca ggctcacacc agagctctga agtccctcat ctccttcctc 720

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cagaacgact tttactggcc atggcaaatt gcagtctacc tgtgcatatc tgtccatccc 840

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<210> 9
<211> 309
<212> PRT
<213> Homo sapiens

<400> 9

Met Ile Thr Phe Leu Pro Ile Ile Phe Ser Ser Leu Val Val Val Thr
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Phe Val Ile Gly Asn Phe Ala Asn Gly Phe Ile Ala Leu Val Asn Ser
20 25 30

Ile Glu Ser Phe Lys Arg Gln Lys Ile Ser Phe Ala Asp Gln Ile Leu
35 40 45

Thr Ala Leu Ala Val Ser Arg Val Gly Leu Leu Trp Val Leu Leu Leu
50 55 60

Asn Trp Tyr Ser Thr Val Leu Asn Pro Ala Phe Asn Ser Val Glu Val
65 70 75 80

Arg Thr Thr Ala Tyr Asn Ile Trp Ala Val Ile Asn His Phe Ser Asn
85 90 95

Trp Leu Ala Thr Thr Leu Ser Ile Phe Tyr Leu Leu Lys Ile Ala Asn
100 105 110

Phe Ser Asn Phe Ile Phe Leu His Leu Lys Arg Arg Val Lys Ser Val
115 120 125

Ile Leu Val Met Leu Leu Gly Pro Leu Leu Phe Leu Ala Cys His Leu
130 135 140

Phe Val Ile Asn Met Asn Glu Ile Val Arg Thr Lys Glu Phe Glu Gly
145 150 155 160

Asn Met Thr Trp Lys Ile Lys Leu Lys Ser Ala Met Tyr Phe Ser Asn
165 170 175

Met Thr Val Thr Met Val Ala Asn Leu Val Pro Phe Thr Leu Thr Leu
180 185 190

Leu Ser Phe Met Leu Leu Ile Cys Ser Leu Cys Lys His Leu Lys Lys
195 200 205

Met Gln Leu Arg Gly Lys Gly Ser Gln Asp Pro Ser Thr Lys Val His
210 215 220

Ile Lys Ala Leu Gln Thr Val Ile Ser Phe Leu Leu Leu Cys Ala Ile
225 230 235 240

Tyr Phe Leu Ser Ile Met Ile Ser Val Trp Ser Phe Gly Ser Leu Glu
245 250 255

Asn Lys Pro Val Phe Met Phe Cys Lys Ala Ile Arg Phe Ser Tyr Pro
260 265 270

Ser Ile His Pro Phe Ile Leu Ile Trp Gly Asn Lys Lys Leu Lys Gln
275 280 285

Thr Phe Leu Ser Val Phe Trp Gln Met Arg Tyr Trp Val Lys Gly Glu
290 295 300

Lys Thr Ser Ser Pro
305

<210> 10

<211> 927
<212> DNA
<213> Homo sapiens

<400> 10
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atctcctttg ctgaccaaat tctcactgct ctggcggtct ccagagttgg tttgctctgg 180

gtattattat taaactggta ttcaactgtg ttgaatccag cttttaatag tgtagaagta 240

agaactactg cttataatat ctgggcagtg atcaaccatt tcagcaactg gcttgctact 300

accctcagca tattttattt gctcaagatt gccaatttct ccaactttat ttttcttcac 360

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gcttgtcatc tttttgtgat aaacatgaat gagattgtgc ggacaaaaga atttgaagga 480

aacatgactt ggaagatcaa attgaagagt gcaatgtact tttcaaatat gactgtaacc 540

atggtagcaa acttagtacc cttcactctg accctactat cttttatgct gttaatctgt 600

tctttgtgta aacatctcaa gaagatgcag ctccgtggta aaggatctca agatcccagc 660

acgaaggtcc acataaaagc tttgcaaact gtgatctcct tcctcttgtt atgtgccatt 720

tactttctgt ccataatgat atcagtttgg agttttggaa gtctggaaaa caaacctgtc 780

ttcatgttct gcaaagctat tagattcagc tatccttcaa tccacccatt catcctgatt 840

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gtgaaaggag agaagacttc atctcca 927

<210> 11
<211> 309
<212> PRT
<213> Homo sapiens

<400> 11

Met Thr Thr Phe Ile Pro Ile Ile Phe Ser Ser Val Val Val Val Leu
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Phe Val Ile Gly Asn Phe Ala Asn Gly Phe Ile Ala Leu Val Asn Ser
            20   25   30

Ile Glu Arg Val Lys Arg Gln Lys Ile Ser Phe Ala Asp Gln Ile Leu
        35   40   45

Thr Ala Leu Ala Val Ser Arg Val Gly Leu Leu Trp Val Leu Leu Leu
    50   55   60

Asn Trp Tyr Ser Thr Val Phe Asn Pro Ala Phe Tyr Ser Val Glu Val
65   70   75   80

Arg Thr Thr Ala Tyr Asn Val Trp Ala Val Thr Gly His Phe Ser Asn
85 90 95

Trp Leu Ala Thr Ser Leu Ser Ile Phe Tyr Leu Leu Lys Ile Ala Asn
100 105 110

Phe Ser Asn Leu Ile Phe Leu His Leu Lys Arg Arg Val Lys Ser Val
115 120 125

Ile Leu Val Met Leu Leu Gly Pro Leu Leu Phe Leu Ala Cys Gln Leu
130 135 140

Phe Val Ile Asn Met Lys Glu Ile Val Arg Thr Lys Glu Tyr Glu Gly
145 150 155 160

Asn Met Thr Trp Lys Ile Lys Leu Arg Ser Ala Val Tyr Leu Ser Asp
165 170 175

Ala Thr Val Thr Thr Leu Gly Asn Leu Val Pro Phe Thr Leu Thr Leu
180 185 190

Leu Cys Phe Leu Leu Leu Ile Cys Ser Leu Cys Lys His Leu Lys Lys
195 200 205

Met Gln Leu His Gly Lys Gly Ser Gln Asp Pro Ser Thr Lys Val His
210 215 220

Ile Lys Ala Leu Gln Thr Val Ile Phe Phe Leu Leu Leu Cys Ala Val
225 230 235 240

Tyr Phe Leu Ser Ile Met Ile Ser Val Trp Ser Phe Gly Ser Leu Glu
245 250 255

Asn Lys Pro Val Phe Met Phe Cys Lys Ala Ile Arg Phe Ser Tyr Pro
260 265 270

Ser Ile His Pro Phe Ile Leu Ile Trp Gly Asn Lys Lys Leu Lys Gln
275 280 285

Thr Phe Leu Ser Val Leu Arg Gln Val Arg Tyr Trp Val Lys Gly Glu
290 295 300

Lys Pro Ser Ser Pro
305

<210> 12

<211> 927
<212> DNA
<213> Homo sapiens

<400> 12
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atctcttttg ctgaccagat tctcactgct ctggcggtct ccagagttgg tttgctctgg 180

gtattattat taaattggta ttcaactgtg tttaatccag ctttttatag tgtagaagta 240

agaactactg cttataatgt ctgggcagta accggccatt tcagcaactg gcttgctact 300

agcctcagca tattttattt gctcaagatt gccaatttct ccaaccttat ttttcttcac 360

ttaaagagga gagttaagag tgtcattctg gtgatgctgt tggggccttt actatttttg 420

gcttgtcaac tttttgtgat aaacatgaaa gagattgtac ggacaaaaga atatgaagga 480

aacatgactt ggaagatcaa attgaggagt gcagtgtacc tttcagatgc gactgtaacc 540

acgctaggaa acttagtgcc cttcactctg accctgctat gtttttgct gttaatctgt 600

tctctgtgta aacatctcaa gaagatgcag ctccatggta aaggatctca agatcccagc 660

accaaggtcc acataaaagc tttgcaaact gtgatctttt tcctcttgtt atgtgccgtt 720

tactttctgt ccataatgat atcagtttgg agttttggga gtctggaaaa caaacctgtc 780

ttcatgttct gcaaagctat tagattcagc tatccttcaa tccacccatt catcctgatt 840

tggggaaaca agaagctaaa gcagactttt ctttcagttt tgcggcaagt gaggtactgg 900

gtgaaaggag agaagccttc atctcca 927

<210> 13
<211> 299
<212> PRT
<213> Homo sapiens

<400> 13

Met Ile Thr Phe Leu Pro Ile Ile Phe Ser Ile Leu Val Val Val Thr
1 5 10 15

Phe Val Ile Gly Asn Phe Ala Asn Gly Phe Ile Ala Leu Val Asn Ser
20 25 30

Thr Glu Trp Val Lys Arg Gln Lys Ile Ser Phe Ala Asp Gln Ile Val
35 40 45

Thr Ala Leu Ala Val Ser Arg Val Gly Leu Leu Trp Val Leu Leu Leu
50 55 60

Asn Trp Tyr Ser Thr Val Leu Asn Pro Ala Phe Cys Ser Val Glu Leu
65 70 75 80

Arg Thr Thr Ala Tyr Asn Ile Trp Ala Val Thr Gly His Phe Ser Asn
85 90 95

Trp Pro Ala Thr Ser Leu Ser Ile Phe Tyr Leu Leu Lys Ile Ala Asn
100 105 110

Phe Ser Asn Leu Ile Phe Leu Arg Leu Lys Arg Arg Val Lys Ser Val
115 120 125

Ile Leu Val Val Leu Leu Gly Pro Leu Leu Phe Leu Ala Cys His Leu
130 135 140

Phe Val Val Asn Met Asn Gln Ile Val Trp Thr Lys Glu Tyr Glu Gly
145 150 155 160

Asn Met Thr Trp Lys Ile Lys Leu Arg Arg Ala Met Tyr Leu Ser Asp
165 170 175

Thr Thr Val Thr Met Leu Ala Asn Leu Val Pro Phe Thr Val Thr Leu
180 185 190

Ile Ser Phe Leu Leu Leu Val Cys Ser Leu Cys Lys His Leu Lys Lys
195 200 205

Met Gln Leu His Gly Lys Gly Ser Gln Asp Pro Ser Thr Lys Val His
210 215 220

Ile Lys Val Leu Gln Thr Val Ile Ser Phe Phe Leu Leu Arg Ala Ile
225 230 235 240

Tyr Phe Val Ser Val Ile Ile Ser Val Trp Ser Phe Lys Asn Leu Glu
245 250 255

Asn Lys Pro Val Phe Met Phe Cys Gln Ala Ile Gly Phe Ser Cys Ser
260 265 270

Ser Ala His Pro Phe Ile Leu Ile Trp Gly Asn Lys Lys Leu Lys Gln
275 280 285

Thr Tyr Leu Ser Val Leu Trp Gln Met Arg Tyr
290 295

<210> 14
<211> 897
<212> DNA
<213> Homo sapiens

<400> 14

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atctcctttg ctgaccaaat tgtcactgct ctggcggtct ccagagttgg tttgctctgg    180
gtgttattat taaattggta ttcaactgtg ttgaatccag ctttttgtag tgtagaatta    240
agaactactg cttataatat ctgggcagta accggccatt tcagcaactg gcctgctact    300
agcctcagca tattttattt gctcaagatt gccaatttct ccaaccttat tttcttcgc     360
ttaaagagga gagttaagag tgtcattctg gtggtgctgt tggggccttt gctatttttg    420
gcttgtcatc tttttgtggt aaacatgaat cagattgtat ggacaaaaga atatgaagga    480
aacatgactt ggaagatcaa attgaggcgt gcaatgtacc tttcagatac gactgtaacc    540
atgctagcaa acttagtacc ctttactgta accctgatat cttttctgct gttagtctgt    600
tctctgtgta aacatctcaa gaagatgcag ctccatggca aaggatctca agatcccagt    660
accaaggtcc acataaaagt tttgcaaact gtgatctcct tcttcttgtt acgtgccatt    720
tactttgtgt ctgtaataat atcagtttgg agttttaaga atctggaaaa caaacctgtc    780
ttcatgttct gccaagctat tggattcagc tgttcttcag cccacccgtt catcctgatt    840
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<210> 15
<211> 299
<212> PRT
<213> Homo sapiens

<400> 15

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Met Ile Thr Phe Leu Pro Ile Ile Phe Ser Ile Leu Ile Val Val Thr
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Phe Val Ile Gly Asn Phe Ala Asn Gly Phe Ile Ala Leu Val Asn Ser
            20                  25                  30

Ile Glu Trp Phe Lys Arg Gln Lys Ile Ser Phe Ala Asp Gln Ile Leu
        35                  40                  45

Thr Ala Leu Ala Val Ser Arg Val Gly Leu Leu Trp Val Leu Val Leu
    50                  55                  60

Asn Trp Tyr Ala Thr Glu Leu Asn Pro Ala Phe Asn Ser Ile Glu Val
65                  70                  75                  80

Arg Ile Thr Ala Tyr Asn Val Trp Ala Val Ile Asn His Phe Ser Asn
                85                  90                  95
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Trp Leu Ala Thr Ser Leu Ser Ile Phe Tyr Leu Leu Lys Ile Ala Asn
100 105 110

Phe Ser Asn Leu Ile Phe Leu His Leu Lys Arg Arg Val Lys Ser Val
115 120 125

Val Leu Val Ile Leu Leu Gly Pro Leu Leu Phe Leu Val Cys His Leu
130 135 140

Phe Val Ile Asn Met Asn Gln Ile Ile Trp Thr Lys Glu Tyr Glu Gly
145 150 155 160

Asn Met Thr Trp Lys Ile Lys Leu Arg Ser Ala Met Tyr Leu Ser Asn
165 170 175

Thr Thr Val Thr Ile Leu Ala Asn Leu Val Pro Phe Thr Leu Thr Leu
180 185 190

Ile Ser Phe Leu Leu Leu Ile Cys Ser Leu Cys Lys His Leu Lys Lys
195 200 205

Met Gln Leu His Gly Lys Gly Ser Gln Asp Pro Ser Met Lys Val His
210 215 220

Ile Lys Ala Leu Gln Thr Val Thr Ser Phe Leu Leu Leu Cys Ala Ile
225 230 235 240

Tyr Phe Leu Ser Ile Ile Met Ser Val Trp Ser Phe Glu Ser Leu Glu
245 250 255

Asn Lys Pro Val Phe Met Phe Cys Glu Ala Ile Ala Phe Ser Tyr Pro
260 265 270

Ser Thr His Pro Phe Ile Leu Ile Trp Gly Asn Lys Lys Leu Lys Gln
275 280 285

Thr Phe Leu Ser Val Leu Trp Gln Met Arg Tyr
290 295

<210> 16
<211> 897
<212> DNA
<213> Homo sapiens

<400> 16
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atctcttttg ctgaccaaat tctcactgct ctggcagtct ccagagttgg tttactctgg 180

gtattagtat taaattggta tgcaactgag ttgaatccag cttttaacag tatagaagta 240

agaattactg cttacaatgt ctgggcagta atcaaccatt tcagcaactg gcttgctact 300

agcctcagca tattttattt gctcaagatt gccaatttct ccaaccttat ttttcttcac 360

ttaaagagga gagttaagag tgttgttctg gtgatactat tggggccttt gctatttttg 420

gtttgtcatc tttttgtgat aaacatgaat cagattatat ggacaaaaga atatgaagga 480

aacatgactt ggaagatcaa actgaggagt gcaatgtacc tttcaaatac aacggtaacc 540

atcctagcaa acttagttcc cttcactctg accctgatat cttttctgct gttaatctgt 600

tctctgtgta aacatctcaa aaagatgcag ctccatggca aaggatctca agatcccagc 660

atgaaggtcc acataaaagc tttgcaaact gtgacctcct tcctcttgtt atgtgccatt 720

tactttctgt ccataatcat gtcagtttgg agttttgaga gtctggaaaa caaacctgtc 780

ttcatgttct gcgaagctat tgcattcagc tatccttcaa cccacccatt catcctgatt 840

tggggaaaca agaagctaaa gcagactttt ctttcagttt tgtggcaaat gaggtac 897

<210> 17
<211> 308
<212> PRT
<213> Homo sapiens

<400> 17

Met Ile Thr Phe Leu Pro Ile Ile Phe Ser Ile Leu Ile Val Val Ile
1 5 10 15

Phe Val Ile Gly Asn Phe Ala Asn Gly Phe Ile Ala Leu Val Asn Ser
20 25 30

Ile Glu Trp Val Lys Arg Gln Lys Ile Ser Phe Val Asp Gln Ile Leu
35 40 45

Thr Ala Leu Ala Val Ser Arg Val Gly Leu Leu Trp Val Leu Leu Leu
50 55 60

His Trp Tyr Ala Thr Gln Leu Asn Pro Ala Phe Tyr Ser Val Glu Val
65 70 75 80

Arg Ile Thr Ala Tyr Asn Val Trp Ala Val Thr Asn His Phe Ser Ser
85 90 95

Trp Leu Ala Thr Ser Leu Ser Met Phe Tyr Leu Leu Arg Ile Ala Asn
100 105 110

Phe Ser Asn Leu Ile Phe Leu Arg Ile Lys Arg Arg Val Lys Ser Val
115 120 125

Val Leu Val Ile Leu Leu Gly Pro Leu Leu Phe Leu Val Cys His Leu
130 135 140

Phe Val Ile Asn Met Asp Glu Thr Val Trp Thr Lys Glu Tyr Glu Gly
145 150 155 160

Asn Val Thr Trp Lys Ile Lys Leu Arg Ser Ala Met Tyr His Ser Asn
165 170 175

Met Thr Leu Thr Met Leu Ala Asn Phe Val Pro Leu Thr Leu Thr Leu
180 185 190

Ile Ser Phe Leu Leu Leu Ile Cys Ser Leu Cys Lys His Leu Lys Lys
195 200 205

Met Gln Leu His Gly Lys Gly Ser Gln Asp Pro Ser Thr Lys Val His
210 215 220

Ile Lys Ala Leu Gln Thr Val Thr Ser Phe Leu Leu Leu Cys Ala Ile
225 230 235 240

Tyr Phe Leu Ser Met Ile Ile Ser Val Cys Asn Phe Gly Arg Leu Glu
245 250 255

Lys Gln Pro Val Phe Met Phe Cys Gln Ala Ile Ile Phe Ser Tyr Pro
260 265 270

Ser Thr His Pro Phe Ile Leu Ile Leu Gly Asn Lys Lys Leu Lys Gln
275 280 285

Ile Phe Leu Ser Val Leu Arg His Val Arg Tyr Trp Val Lys Asp Arg
290 295 300

Ser Leu Arg Leu
305

<210> 18
<211> 926
<212> DNA
<213> Homo sapiens

<400> 18
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atctcctttg ttgaccaaat tctcactgct ctggcggtct ccagagttgg tttgctctgg 180

gtgttattac tacattggta tgcaactcag ttgaatccag ctttttatag tgtagaagta 240

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atgctagcaa actttgtacc cctcactctg accctgatat cttttctgct gttaatctgt 600

tctctgtgta aacatctcaa gaagatgcag ctccatggca aaggatctca agatcccagc 660

accaaggtcc acataaaagc tttgcaaact gtgacctcct ttcttctgtt atgtgccatt 720

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gtgaaagaca gaagccttcg tctcca 926

<210> 19
<211> 298
<212> PRT
<213> Homo sapiens

<400> 19

Met Met Cys Phe Leu Leu Ile Ile Ser Ser Ile Leu Val Val Phe Ala
1 5 10 15

Phe Val Leu Gly Asn Val Ala Asn Gly Phe Ile Ala Leu Val Asn Val
20 25 30

Ile Asp Trp Val Asn Thr Arg Lys Ile Ser Ser Ala Glu Gln Ile Leu
35 40 45

Thr Ala Leu Val Val Ser Arg Ile Gly Leu Leu Trp Val Met Leu Phe
50 55 60

Leu Trp Tyr Ala Thr Val Phe Asn Ser Ala Leu Tyr Gly Leu Glu Val
65 70 75 80

Arg Ile Val Ala Ser Asn Ala Trp Ala Val Thr Asn His Phe Ser Met
85 90 95

Trp Leu Ala Ala Ser Leu Ser Ile Phe Cys Leu Leu Lys Ile Ala Asn
100 105 110

Phe Ser Asn Leu Ile Ser Leu His Leu Lys Lys Arg Ile Lys Ser Val
115 120 125

Val Leu Val Ile Leu Leu Gly Pro Leu Val Phe Leu Ile Cys Asn Leu
130 135 140

Ala Val Ile Thr Met Asp Glu Arg Val Trp Thr Lys Glu Tyr Glu Gly
145 150 155 160

Asn Val Thr Trp Lys Ile Lys Leu Arg Asn Ala Ile His Leu Ser Ser
165 170 175

Leu Thr Val Thr Thr Leu Ala Asn Leu Ile Pro Phe Thr Leu Ser Leu
180 185 190

Ile Cys Phe Leu Leu Leu Ile Cys Ser Leu Cys Lys His Leu Lys Lys
195 200 205

Met Arg Leu His Ser Lys Gly Ser Gln Asp Pro Ser Thr Lys Val His
210 215 220

Ile Lys Ala Leu Gln Thr Val Thr Ser Phe Leu Met Leu Phe Ala Ile
225 230 235 240

Tyr Phe Leu Cys Ile Ile Thr Ser Thr Trp Asn Leu Arg Thr Gln Gln
245 250 255

Ser Lys Leu Val Leu Leu Leu Cys Gln Thr Val Ala Ile Met Tyr Pro
260 265 270

Ser Phe His Ser Phe Ile Leu Ile Met Gly Ser Arg Lys Leu Lys Gln
275 280 285

Thr Phe Leu Ser Val Leu Trp Gln Met Thr
290 295

<210> 20
<211> 897
<212> DNA
<213> Homo sapiens

<400> 20
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atctcctcag ctgagcaaat tctcactgct ctggtggtct ccagaattgg tttactctgg 180

gtcatgttat tcctttggta tgcaactgtg tttaattctg ctttatatgg tttagaagta 240

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agcctcagca tattttgttt gctcaagatt gccaatttct ccaaccttat ttctctccac 360

ctaaagaaga gaattaagag tgttgttctg gtgatactgt tggggccctt ggtatttttg 420

atttgtaatc ttgctgtgat aaccatggat gagagagtgt ggacaaaaga atatgaagga 480

aatgtgactt ggaagatcaa attgaggaat gcaatacacc tttcaagctt gactgtaact 540

actctagcaa acctcatacc ctttactctg agcctaatat gttttctgct gttaatctgt 600

tctctttgta aacatctcaa gaagatgcgg ctccatagca aaggatctca agatcccagc 660

accaaggtcc atataaaagc tttgcaaact gtgacctcct tcctcatgtt atttgccatt 720

tactttctgt gtataatcac atcaacttgg aatcttagga cacagcagag caaacttgta 780

ctcctgcttt gccaaactgt tgcaatcatg tatccttcat tccactcatt catcctgatt 840

atgggaagta ggaagctaaa acagaccttt ctttcagttt tgtggcagat gacacgc 897

<210> 21
<211> 309
<212> PRT
<213> Homo sapiens

<400> 21

Met Met Ser Phe Leu His Ile Val Phe Ser Ile Leu Val Val Val Ala
1 5 10 15

Phe Ile Leu Gly Asn Phe Ala Asn Gly Phe Ile Ala Leu Ile Asn Phe
20 25 30

Ile Ala Trp Val Lys Arg Gln Lys Ile Ser Ser Ala Asp Gln Ile Ile
35 40 45

Ala Ala Leu Ala Val Ser Lys Val Gly Leu Leu Trp Val Ile Leu Leu
50 55 60

His Trp Tyr Ser Thr Val Leu Asn Pro Thr Ser Ser Asn Leu Lys Val
65 70 75 80

Ile Ile Phe Ile Ser Asn Ala Trp Ala Val Thr Asn His Phe Ser Ile
85 90 95

Trp Leu Ala Thr Ser Leu Ser Ile Phe Tyr Leu Leu Lys Ile Val Asn
100 105 110

Phe Ser Arg Leu Ile Phe His His Leu Lys Arg Lys Ala Lys Ser Val
115 120 125

Val Leu Val Ile Val Leu Gly Ser Leu Phe Phe Leu Val Cys His Leu
130 135 140

Val Met Lys His Thr Tyr Ile Asn Val Trp Thr Glu Glu Cys Glu Gly
145 150 155 160

Asn Val Thr Trp Lys Ile Lys Leu Arg Asn Ala Met His Leu Ser Asn
165 170 175

Leu Thr Val Ala Met Leu Ala Asn Leu Ile Pro Phe Thr Leu Thr Leu
180 185 190

Ile Ser Phe Leu Leu Leu Ile Tyr Ser Leu Cys Lys His Leu Lys Lys
195 200 205

Met Gln Leu His Gly Lys Gly Ser Gln Asp Pro Ser Thr Lys Ile His
210 215 220

Ile Lys Ala Leu Gln Thr Val Thr Ser Phe Leu Ile Leu Leu Ala Ile
225 230 235 240

Tyr Phe Leu Cys Leu Ile Ile Ser Phe Trp Asn Phe Lys Met Arg Pro
245 250 255

Lys Glu Ile Val Leu Met Leu Cys Gln Ala Phe Gly Ile Ile Tyr Pro
260 265 270

Ser Phe His Ser Phe Ile Leu Ile Trp Gly Asn Lys Thr Leu Lys Gln
275 280 285

Thr Phe Leu Ser Val Leu Trp Gln Val Thr Cys Trp Ala Lys Gly Gln
290 295 300

Asn Gln Ser Thr Pro
305

<210> 22
<211> 927
<212> DNA
<213> Homo sapiens

<400> 22
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atctcctcag ctgatcaaat tattgctgct ctggcagtct ccaaagttgg tttgctctgg 180

gtaatattat tacattggta ttcaactgtg ttgaatccaa cttcatctaa tttaaaagta 240

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agcctcagca tattttattt gctcaagatc gtcaatttct ccagacttat ttttcatcac 360

ttaaaaagga aggctaagag tgtagttctg gtgatagtgt tggggtcttt gttctttttg 420

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gtttgtcacc ttgtgatgaa acacacgtat ataaatgtgt ggacagaaga atgtgaagga     480
aacgtaactt ggaagatcaa actgaggaat gcaatgcacc tttccaactt gactgtagcc     540
atgctagcaa acttgatacc attcactctg accctgatat cttttctgct gttaatctac     600
tctctgtgta aacatctgaa gaagatgcag ctccatggca aaggatctca agatcccagc     660
accaagatcc acataaaagc tctgcaaact gtgacctcct tcctcatatt acttgccatt     720
tactttctgt gtctaatcat atcgttttgg aattttaaga tgcgaccaaa agaaattgtc     780
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gcaaaaggac agaaccagtc aactcca                                         927
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<210>  23
<211>  299
<212>  PRT
<213>  Homo sapiens

<400>  23

Met Ile Thr Phe Leu Tyr Ile Phe Phe Ser Ile Leu Ile Met Val Leu
1               5                   10                  15

Phe Val Leu Gly Asn Phe Ala Asn Gly Phe Ile Ala Leu Val Asn Phe
            20                  25                  30

Ile Asp Trp Val Lys Arg Lys Lys Ile Ser Ser Ala Asp Gln Ile Leu
        35                  40                  45

Thr Ala Leu Ala Val Ser Arg Ile Gly Leu Leu Trp Ala Leu Leu Leu
    50                  55                  60

Asn Trp Tyr Leu Thr Val Leu Asn Pro Ala Phe Tyr Ser Val Glu Leu
65                  70                  75                  80

Arg Ile Thr Ser Tyr Asn Ala Trp Val Val Thr Asn His Phe Ser Met
                85                  90                  95

Trp Leu Ala Ala Asn Leu Ser Ile Phe Tyr Leu Leu Lys Ile Ala Asn
            100                 105                 110

Phe Ser Asn Leu Leu Phe Leu His Leu Lys Arg Arg Val Arg Ser Val
        115                 120                 125

Ile Leu Val Ile Leu Leu Gly Thr Leu Ile Phe Leu Val Cys His Leu
    130                 135                 140
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Leu Val Ala Asn Met Asp Glu Ser Met Trp Ala Glu Glu Tyr Glu Gly
145 150 155 160

Asn Met Thr Gly Lys Met Lys Leu Arg Asn Thr Val His Leu Ser Tyr
165 170 175

Leu Thr Val Thr Thr Leu Trp Ser Phe Ile Pro Phe Thr Leu Ser Leu
180 185 190

Ile Ser Phe Leu Met Leu Ile Cys Ser Leu Tyr Lys His Leu Lys Lys
195 200 205

Met Gln Leu His Gly Glu Gly Ser Gln Asp Leu Ser Thr Lys Val His
210 215 220

Ile Lys Ala Leu Gln Thr Leu Ile Ser Phe Leu Leu Leu Cys Ala Ile
225 230 235 240

Phe Phe Leu Phe Leu Ile Val Ser Val Trp Ser Pro Arg Arg Leu Arg
245 250 255

Asn Asp Pro Val Val Met Val Ser Lys Ala Val Gly Asn Ile Tyr Leu
260 265 270

Ala Phe Asp Ser Phe Ile Leu Ile Trp Arg Thr Lys Lys Leu Lys His
275 280 285

Thr Phe Leu Leu Ile Leu Cys Gln Ile Arg Cys
290 295

<210> 24
<211> 897
<212> DNA
<213> Homo sapiens

<400> 24
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aactttgcca atggcttcat agcactggta aatttcattg actgggtgaa gagaaaaaag 120

atctcctcag ctgaccaaat tctcactgct ctggcggtct ccagaattgg tttgctctgg 180

gcattattat taaattggta tttaactgtg ttgaatccag ctttttatag tgtagaatta 240

agaattactt cttataatgc ctgggttgta accaaccatt tcagcatgtg gcttgctgct 300

aacctcagca tattttattt gctcaagatt gccaatttct ccaaccttct ttttcttcat 360

ttaaagagga gagttaggag tgtcattctg gtgatactgt tggggacttt gatatttttg 420

gtttgtcatc ttcttgtggc aaacatggat gagagtatgt gggcagaaga atatgaagga 480

aacatgactg ggaagatgaa attgaggaat acagtacatc tttcatattt gactgtaact 540

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accaaggtcc acataaaagc tttgcaaact ctgatctcct tcctcttgtt atgtgccatt    720
ttctttctat tcctaatcgt ttcggtttgg agtcctagga ggctgcggaa tgacccagtt    780
gtcatggtta gcaaggctgt tggaaacata tatcttgcat tcgactcatt catcctaatt    840
tggagaacca agaagctaaa acacaccttt cttttgattt tgtgtcagat taggtgc       897
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<210>  25
<211>  299
<212>  PRT
<213>  Homo sapiens

<400>  25

Met Leu Glu Ser His Leu Ile Ile Tyr Phe Leu Leu Ala Val Ile Gln
1               5                   10                  15

Phe Leu Leu Gly Ile Phe Thr Asn Gly Ile Ile Val Val Val Asn Gly
            20                  25                  30

Ile Asp Leu Ile Lys His Arg Lys Met Ala Pro Leu Asp Leu Leu Leu
        35                  40                  45

Ser Cys Leu Ala Val Ser Arg Ile Phe Leu Gln Leu Phe Ile Phe Tyr
    50                  55                  60

Val Asn Val Ile Val Ile Phe Phe Ile Glu Phe Ile Met Cys Ser Ala
65                  70                  75                  80

Asn Cys Ala Ile Leu Leu Phe Ile Asn Glu Leu Glu Leu Trp Leu Ala
                85                  90                  95

Thr Trp Leu Gly Val Phe Tyr Cys Ala Lys Val Ala Ser Val Arg His
            100                 105                 110

Pro Leu Phe Ile Trp Leu Lys Met Arg Ile Ser Lys Leu Val Pro Trp
        115                 120                 125

Met Ile Leu Gly Ser Leu Leu Tyr Val Ser Met Ile Cys Val Phe His
    130                 135                 140

Ser Lys Tyr Ala Gly Phe Met Val Pro Tyr Phe Leu Arg Lys Phe Phe
145                 150                 155                 160

Ser Gln Asn Ala Thr Ile Gln Lys Glu Asp Thr Leu Ala Ile Gln Ile
                165                 170                 175
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Phe Ser Phe Val Ala Glu Phe Ser Val Pro Leu Leu Ile Phe Leu Phe
        180                 185                 190

Ala Val Leu Leu Leu Ile Phe Ser Leu Gly Arg His Thr Arg Gln Met
    195                 200                 205

Arg Asn Thr Val Ala Gly Ser Arg Val Pro Gly Arg Gly Ala Pro Ile
    210                 215                 220

Ser Ala Leu Leu Ser Ile Leu Ser Phe Leu Ile Leu Tyr Phe Ser His
225                 230                 235                 240

Cys Met Ile Lys Val Phe Leu Ser Ser Leu Lys Phe His Ile Arg Arg
                245                 250                 255

Phe Ile Phe Leu Phe Phe Ile Leu Val Ile Gly Ile Tyr Pro Ser Gly
        260                 265                 270

His Ser Leu Ile Leu Ile Leu Gly Asn Pro Lys Leu Lys Gln Asn Ala
    275                 280                 285

Lys Lys Phe Leu Leu His Ser Lys Cys Cys Gln
    290                 295


<210>  26
<211>  897
<212>  DNA
<213>  Homo sapiens

<400>  26
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atggctccgc tggatctcct tctttcttgt ctggcagttt ctagaatttt tctgcagttg     180

ttcatcttct acgttaatgt gattgttatc ttcttcatag aattcatcat gtgttctgcg     240

aattgtgcaa ttctcttatt tataaatgaa ttggaacttt ggcttgccac atggctcggc     300

gttttctatt gtgccaaggt tgccagcgtc cgtcacccac tcttcatctg gttgaagatg     360

aggatatcca agctggtccc atggatgatc ctggggtctc tgctatatgt atctatgatt     420

tgtgttttcc atagcaaata tgcagggttt atggtcccat acttcctaag gaaatttttc     480

tcccaaaatg ccacaattca aaaagaagat acactggcta tacagatttt ctcttttgtt     540

gctgagttct cagtgccatt gcttatcttc ctttttgctg ttttgctctt gattttctct     600

ctggggaggc acacccggca aatgagaaac acagtggccg gcagcagggt tcctggcagg     660

ggtgcaccca tcagcgcgtt gctgtctatc ctgtccttcc tgatcctcta cttctcccac     720
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tgcatgataa aagtttttct ctcttctcta aagtttcaca tcagaaggtt catctttctg 780

ttcttcatcc ttgtgattgg tatatacccт tctggacact ctctcatctt aattttagga 840

aatcctaaat tgaaacaaaa tgcaaaaaag ttcctcctcc acagtaagtg ctgtcag 897

<210> 27
<211> 299
<212> PRT
<213> Homo sapiens

<400> 27

Met Leu Arg Leu Phe Tyr Phe Ser Ala Ile Ile Ala Ser Val Ile Leu
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Asn Phe Val Gly Ile Ile Met Asn Leu Phe Ile Thr Val Val Asn Cys
20 25 30

Lys Thr Trp Val Lys Ser His Arg Ile Ser Ser Ser Asp Arg Ile Leu
35 40 45

Phe Ser Leu Gly Ile Thr Arg Phe Leu Met Leu Gly Leu Phe Leu Val
50 55 60

Asn Thr Ile Tyr Phe Val Ser Ser Asn Thr Glu Arg Ser Val Tyr Leu
65 70 75 80

Ser Ala Phe Phe Val Leu Cys Phe Met Phe Leu Asp Ser Ser Ser Val
85 90 95

Trp Phe Val Thr Leu Leu Asn Ile Leu Tyr Cys Val Lys Ile Thr Asn
100 105 110

Phe Gln His Ser Val Phe Leu Leu Leu Lys Arg Asn Ile Ser Pro Lys
115 120 125

Ile Pro Arg Leu Leu Leu Ala Cys Val Leu Ile Ser Ala Phe Thr Thr
130 135 140

Cys Leu Tyr Ile Thr Leu Ser Gln Ala Ser Pro Phe Pro Glu Leu Val
145 150 155 160

Thr Thr Arg Asn Asn Thr Ser Phe Asn Ile Ser Glu Gly Ile Leu Ser
165 170 175

Leu Val Val Ser Leu Val Leu Ser Ser Ser Leu Gln Phe Ile Ile Asn
180 185 190

Val Thr Ser Ala Ser Leu Leu Ile His Ser Leu Arg Arg His Ile Gln
195 200 205

Lys Met Gln Lys Asn Ala Thr Gly Phe Trp Asn Pro Gln Thr Glu Ala
210 215 220

His Val Gly Ala Met Lys Leu Met Val Tyr Phe Leu Ile Leu Tyr Ile
225 230 235 240

Pro Tyr Ser Val Ala Thr Leu Val Gln Tyr Leu Pro Phe Tyr Ala Gly
245 250 255

Met Asp Met Gly Thr Lys Ser Ile Cys Leu Ile Phe Ala Thr Leu Tyr
260 265 270

Ser Pro Gly His Ser Val Leu Ile Ile Ile Thr His Pro Lys Leu Lys
275 280 285

Thr Thr Ala Lys Lys Ile Leu Cys Phe Lys Lys
290 295

<210> 28
<211> 897
<212> DNA
<213> Homo sapiens

<400> 28
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atctcctctt ctgataggat tctgttcagc ctgggcatca ccaggtttct tatgctggga 180

ctatttctgg tgaacaccat ctacttcgtc tcttcaaata cggaaaggtc agtctacctg 240

tctgcttttt ttgtgttgtg tttcatgttt ttggactcga gcagtgtctg gtttgtgacc 300

ttgctcaata tcttgtactg tgtgaagatt actaacttcc aacactcagt gtttctcctg 360

ctgaagcgga atatctcccc aaagatcccc aggctgctgc tggcctgtgt gctgatttct 420

gctttcacca cttgcctgta catcacgctt agccaggcat caccttttcc tgaacttgtg 480

actacgagaa ataacacatc atttaatatc agtgagggca tcttgtcttt agtggtttct 540

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cagacggaag ctcatgtagg tgctatgaag ctgatggtct atttcctcat cctctacatt 720

ccatattcag ttgctaccct ggtccagtat ctcccctttt atgcagggat ggatatgggg 780

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<210> 29
<211> 299
<212> PRT
<213> Homo sapiens

<400> 29

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Met Leu Ser Ala Gly Leu Gly Leu Leu Met Leu Val Ala Val Val Glu
1               5                   10                  15

Phe Leu Ile Gly Leu Ile Gly Asn Gly Ser Leu Val Val Trp Ser Phe
            20                  25                  30

Arg Glu Trp Ile Arg Lys Phe Asn Trp Ser Ser Tyr Asn Leu Ile Ile
        35                  40                  45

Leu Gly Leu Ala Gly Cys Arg Phe Leu Leu Gln Trp Leu Ile Ile Leu
    50                  55                  60

Asp Leu Ser Leu Phe Pro Leu Phe Gln Ser Ser Arg Trp Leu Arg Tyr
65                  70                  75                  80

Leu Ser Ile Phe Trp Val Leu Val Ser Gln Ala Ser Leu Trp Phe Ala
                85                  90                  95

Thr Phe Leu Ser Val Phe Tyr Cys Lys Lys Ile Thr Thr Phe Asp Arg
            100                 105                 110

Pro Ala Tyr Leu Trp Leu Lys Gln Arg Ala Tyr Asn Leu Ser Leu Trp
        115                 120                 125

Cys Leu Leu Gly Tyr Phe Ile Ile Asn Leu Leu Leu Thr Val Gln Ile
    130                 135                 140

Gly Leu Thr Phe Tyr His Pro Pro Gln Gly Asn Ser Ser Ile Arg Tyr
145                 150                 155                 160

Pro Phe Glu Ser Trp Gln Tyr Leu Tyr Ala Phe Gln Leu Asn Ser Gly
                165                 170                 175

Ser Tyr Leu Pro Leu Val Val Phe Leu Val Ser Ser Gly Met Leu Ile
            180                 185                 190

Val Ser Leu Tyr Thr His His Lys Lys Met Lys Val His Ser Ala Gly
        195                 200                 205

Arg Arg Asp Val Arg Ala Lys Ala His Ile Thr Ala Leu Lys Ser Leu
    210                 215                 220
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Gly Cys Phe Leu Leu Leu His Leu Val Tyr Ile Met Ala Ser Pro Phe
225 230 235 240

Ser Ile Thr Ser Lys Thr Tyr Pro Pro Asp Leu Thr Ser Val Phe Ile
245 250 255

Trp Glu Thr Leu Met Ala Ala Tyr Pro Ser Leu His Ser Leu Ile Leu
260 265 270

Ile Met Gly Ile Pro Arg Val Lys Gln Thr Cys Gln Lys Ile Leu Trp
275 280 285

Lys Thr Val Cys Ala Arg Arg Cys Trp Gly Pro
290 295

<210> 30
<211> 897
<212> DNA
<213> Homo sapiens

<400> 30
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tggtcctcat ataacctcat tatcctgggc ctggctggct gccgatttct cctgcagtgg 180

ctgatcattt tggacttaag cttgtttcca cttttccaga gcagccgttg gcttcgctat 240

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gtcttctatt gcaagaagat cacgaccttc gatcgcccgg cctacttgtg gctgaagcag 360

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aagatgaagg tccattcagc tggtaggagg gatgtccggg ccaaggctca catcactgcg 660

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atggcagcct atccttctct tcattctctc atattgatca tggggattcc tagggtgaag 840

cagacttgtc agaagatcct gtggaagacg gtgtgtgctc ggagatgctg gggccca 897

<210> 31
<211> 318
<212> PRT
<213> Homo sapiens

<400> 31

Met Ala Asp Lys Val Gln Thr Thr Leu Leu Phe Leu Ala Val Gly Glu
1 5 10 15

Phe Ser Val Gly Ile Leu Gly Asn Ala Phe Ile Gly Leu Val Asn Cys
20 25 30

Met Asp Trp Val Lys Lys Arg Lys Ile Ala Ser Ile Asp Leu Ile Leu
35 40 45

Thr Ser Leu Ala Ile Ser Arg Ile Cys Leu Leu Cys Val Ile Leu Leu
50 55 60

Asp Cys Phe Ile Leu Val Leu Tyr Pro Asp Val Tyr Ala Thr Gly Lys
65 70 75 80

Glu Met Arg Ile Ile Asp Phe Phe Trp Thr Leu Thr Asn His Leu Ser
85 90 95

Ile Trp Phe Ala Thr Cys Leu Ser Ile Tyr Tyr Phe Phe Lys Ile Gly
100 105 110

Asn Phe Phe His Pro Leu Phe Leu Trp Met Lys Trp Arg Ile Asp Arg
115 120 125

Val Ile Ser Trp Ile Leu Leu Gly Cys Val Val Leu Ser Val Phe Ile
130 135 140

Ser Leu Pro Ala Thr Glu Asn Leu Asn Ala Asp Phe Arg Phe Cys Val
145 150 155 160

Lys Ala Lys Arg Lys Thr Asn Leu Thr Trp Ser Cys Arg Val Asn Lys
165 170 175

Thr Gln His Ala Ser Thr Lys Leu Phe Leu Asn Leu Ala Thr Leu Leu
180 185 190

Pro Phe Cys Val Cys Leu Met Ser Phe Phe Leu Leu Ile Leu Ser Leu
195 200 205

Arg Arg His Ile Arg Arg Met Gln Leu Ser Ala Thr Gly Cys Arg Asp
210 215 220

Pro Ser Thr Glu Ala His Val Arg Ala Leu Lys Ala Val Ile Ser Phe
225 230 235 240

Leu Leu Leu Phe Ile Ala Tyr Tyr Leu Ser Phe Leu Ile Ala Thr Ser
245 250 255

Ser Tyr Phe Met Pro Glu Thr Glu Leu Ala Val Ile Phe Gly Glu Ser
260 265 270

Ile Ala Leu Ile Tyr Pro Ser Ser His Ser Phe Ile Leu Ile Leu Gly
275 280 285

Asn Asn Lys Leu Arg His Ala Ser Leu Lys Val Ile Trp Lys Val Met
290 295 300

Ser Ile Leu Lys Gly Arg Lys Phe Gln Gln His Lys Gln Ile
305 310 315

<210> 32
<211> 954
<212> DNA
<213> Homo sapiens

<400> 32
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attgcctcca ttgatttaat cctcacaagt ctggccatat ccagaatttg tctattgtgc 180

gtaatactat tagattgttt tatattggtg ctatatccag atgtctatgc cactggtaaa 240

gaaatgagaa tcattgactt cttctggaca ctaaccaatc atttaagtat ctggtttgca 300

acctgcctca gcatttacta tttcttcaag ataggtaatt tctttcaccc acttttcctc 360

tggatgaagt ggagaattga cagggtgatt tcctggattc tactggggtg cgtggttctc 420

tctgtgttta ttagccttcc agccactgag aatttgaacg ctgatttcag gttttgtgtg 480

aaggcaaaga ggaaaacaaa cttaacttgg agttgcagag taaataaaac tcaacatgct 540

tctaccaagt tatttctcaa cctggcaacg ctgctcccct tttgtgtgtg cctaatgtcc 600

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cttctcctct ttattgccta ctatttgtcc tttctcattg ccacctccag ctactttatg 780

ccagagacgg aattagctgt gatttttggt gagtccatag ctctaatcta cccctcaagt 840

cattcattta tcctaatact ggggaacaat aaattaagac atgcatctct aaaggtgatt 900

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<210> 33
<211> 309
<212> PRT
<213> Homo sapiens

<400> 33

Met Phe Ser Pro Ala Asp Asn Ile Phe Ile Ile Leu Ile Thr Gly Glu
1 5 10 15

Phe Ile Leu Gly Ile Leu Gly Asn Gly Tyr Ile Ala Leu Val Asn Trp
20 25 30

Ile Asp Trp Ile Lys Lys Lys Lys Ile Ser Thr Val Asp Tyr Ile Leu
35 40 45

Thr Asn Leu Val Ile Ala Arg Ile Cys Leu Ile Ser Val Met Val Val
50 55 60

Asn Gly Ile Val Ile Val Leu Asn Pro Asp Val Tyr Thr Lys Asn Lys
65 70 75 80

Gln Gln Ile Val Ile Phe Thr Phe Trp Thr Phe Ala Asn Tyr Leu Asn
85 90 95

Met Trp Ile Thr Thr Cys Leu Asn Val Phe Tyr Phe Leu Lys Ile Ala
100 105 110

Ser Ser Ser His Pro Leu Phe Leu Trp Leu Lys Trp Lys Ile Asp Met
115 120 125

Val Val His Trp Ile Leu Leu Gly Cys Phe Ala Ile Ser Leu Leu Val
130 135 140

Ser Leu Ile Ala Ala Ile Val Leu Ser Cys Asp Tyr Arg Phe His Ala
145 150 155 160

Ile Ala Lys His Lys Arg Asn Ile Thr Glu Met Phe His Val Ser Lys
165 170 175

Ile Pro Tyr Phe Glu Pro Leu Thr Leu Phe Asn Leu Phe Ala Ile Val
180 185 190

Pro Phe Ile Val Ser Leu Ile Ser Phe Phe Leu Leu Val Arg Ser Leu
195 200 205

Trp Arg His Thr Lys Gln Ile Lys Leu Tyr Ala Thr Gly Ser Arg Asp
210 215 220

Pro Ser Thr Glu Val His Val Arg Ala Ile Lys Thr Met Thr Ser Phe
225 230 235 240

Ile Phe Phe Phe Phe Leu Tyr Tyr Ile Ser Ser Ile Leu Met Thr Phe
245 250 255

Ser Tyr Leu Met Thr Lys Tyr Lys Leu Ala Val Glu Phe Gly Glu Ile
260 265 270

Ala Ala Ile Leu Tyr Pro Leu Gly His Ser Leu Ile Leu Ile Val Leu
275 280 285

Asn Asn Lys Leu Arg Gln Thr Phe Val Arg Met Leu Thr Cys Arg Lys
290 295 300

Ile Ala Cys Met Ile
305

<210> 34
<211> 927
<212> DNA
<213> Homo sapiens

<400> 34
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atttccacag ttgactacat ccttaccaat ttagttatcg ccagaatttg tttgatcagt 180

gtaatggttg taaatggcat tgtaatagta ctgaacccag atgtttatac aaaaaataaa 240

caacagatag tcatttttac cttctggaca tttgccaact acttaaatat gtggattacc 300

acctgcctta atgtcttcta ttttctgaag atagccagtt cctctcatcc actttttctc 360

tggctgaagt ggaaaattga tatggtggtg cactggatcc tgctgggatg ctttgccatt 420

tccttgttgg tcagccttat agcagcaata gtactgagtt gtgattatag gtttcatgca 480

attgccaaac ataaaagaaa cattactgaa atgttccatg tgagtaaaat accatacttt 540

gaacccttaa ctctctttaa cctgtttgca attgtcccat ttattgtgtc actgatatca 600

tttttccttt tagtaagatc tttatggaga cataccaagc aaataaaact ctatgctacc 660

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acaaaataca agttagctgt ggagtttgga gagattgcag caattctcta ccccttgggt 840

cactcactta ttttaattgt tttaaataat aaactgaggc agacatttgt cagaatgctg 900

acatgtagaa aaattgcctg catgata 927

<210> 35
<211> 312
<212> PRT
<213> Homo sapiens

<400> 35

Met Pro Ser Ala Ile Glu Ala Ile Tyr Ile Ile Leu Ile Ala Gly Glu
1 5 10 15

Leu Thr Ile Gly Ile Trp Gly Asn Gly Phe Ile Val Leu Val Asn Cys
20 25 30

Ile Asp Trp Leu Lys Arg Arg Asp Ile Ser Leu Ile Asp Ile Ile Leu
35 40 45

Ile Ser Leu Ala Ile Ser Arg Ile Cys Leu Leu Cys Val Ile Ser Leu
50 55 60

Asp Gly Phe Phe Met Leu Leu Phe Pro Gly Thr Tyr Gly Asn Ser Val
65 70 75 80

Leu Val Ser Ile Val Asn Val Val Trp Thr Phe Ala Asn Asn Ser Ser
85 90 95

Leu Trp Phe Thr Ser Cys Leu Ser Ile Phe Tyr Leu Leu Lys Ile Ala
100 105 110

Asn Ile Ser His Pro Phe Phe Phe Trp Leu Lys Leu Lys Ile Asn Lys
115 120 125

Val Met Leu Ala Ile Leu Leu Gly Ser Phe Leu Ile Ser Leu Ile Ile
130 135 140

Ser Val Pro Lys Asn Asp Asp Met Trp Tyr His Leu Phe Lys Val Ser
145 150 155 160

His Glu Glu Asn Ile Thr Trp Lys Phe Lys Val Ser Lys Ile Pro Gly
165 170 175

Thr Phe Lys Gln Leu Thr Leu Asn Leu Gly Val Met Val Pro Phe Ile
180 185 190

Leu Cys Leu Ile Ser Phe Phe Leu Leu Leu Phe Ser Leu Val Arg His
195 200 205

Thr Lys Gln Ile Arg Leu His Ala Thr Gly Phe Arg Asp Pro Ser Thr
210 215 220

Glu Ala His Met Arg Ala Ile Lys Ala Val Ile Ile Phe Leu Leu Leu
225 230 235 240

Leu Ile Val Tyr Tyr Pro Val Phe Leu Val Met Thr Ser Ser Ala Leu
245 250 255

Ile Pro Gln Gly Lys Leu Val Leu Met Ile Gly Asp Ile Val Thr Val
260 265 270

Ile Phe Pro Ser Ser His Ser Phe Ile Leu Ile Met Gly Asn Ser Lys
275 280 285

Leu Arg Glu Ala Phe Leu Lys Met Leu Arg Phe Val Lys Cys Phe Leu
290 295 300

Arg Arg Arg Lys Pro Phe Val Pro
305 310

<210> 36
<211> 936
<212> DNA
<213> Homo sapiens

<400> 36
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atttggggaa atggattcat tgtactagtt aactgcattg actggctcaa aagaagagat 120

atttccttga ttgacatcat cctgatcagc ttggccatct ccagaatctg tctgctgtgt 180

gtaatatcat tagatggctt ctttatgctg ctctttccag gtacatatgg caatagcgtg 240

ctagtaagca ttgtgaatgt tgtctggaca tttgccaata attcaagtct ctggtttact 300

tcttgcctca gtatcttcta tttactcaag atagccaata tatcgcaccc atttttcttc 360

tggctgaagc taaagatcaa caaggtcatg cttgcgattc ttctggggtc ctttcttatc 420

tctttaatta ttagtgttcc aaagaatgat gatatgtggt atcacctttt caaagtcagt 480

catgaagaaa acattacttg gaaattcaaa gtgagtaaaa ttccaggtac tttcaaacag 540

ttaaccctga acctgggggt gatggttccc tttatccttt gcctgatctc atttttcttg 600

ttacttttct ccctagttag acacaccaag cagattcgac tgcatgctac agggttcaga 660

gaccccagta cagaggccca catgagggcc ataaaggcag tgatcatctt tctgctcctc 720

ctcatcgtgt actacccagt ctttcttgtt atgacctcta gcgctctgat tcctcaggga 780

aaattagtgt tgatgattgg tgacatagta actgtcattt tcccatcaag ccattcattc 840

attctaatta tgggaaatag caagttgagg gaagcttttc tgaagatgtt aagatttgtg 900

aagtgtttcc ttagaagaag aaagcctttt gttcca 936

<210> 37
<211> 307
<212> PRT
<213> Homo sapiens

<400> 37

Met Leu Arg Val Val Glu Gly Ile Phe Ile Phe Val Val Val Ser Glu
1 5 10 15

Ser Val Phe Gly Val Leu Gly Asn Gly Phe Ile Gly Leu Val Asn Cys
20 25 30

Ile Asp Cys Ala Lys Asn Lys Leu Ser Thr Ile Gly Phe Ile Leu Thr
35 40 45

Gly Leu Ala Ile Ser Arg Ile Phe Leu Ile Trp Ile Ile Ile Thr Asp
50 55 60

Gly Phe Ile Gln Ile Phe Ser Pro Asn Ile Tyr Ala Ser Gly Asn Leu
65 70 75 80

Ile Glu Tyr Ile Ser Tyr Phe Trp Val Ile Gly Asn Gln Ser Ser Met
85 90 95

Trp Phe Ala Thr Ser Leu Ser Ile Phe Tyr Phe Leu Lys Ile Ala Asn
100 105 110

Phe Ser Asn Tyr Ile Phe Leu Trp Leu Lys Ser Arg Thr Asn Met Val
115 120 125

Leu Pro Phe Met Ile Val Phe Leu Leu Ile Ser Ser Leu Leu Asn Phe
130 135 140

Ala Tyr Ile Ala Lys Ile Leu Asn Asp Tyr Lys Met Lys Asn Asp Thr
145 150 155 160

Val Trp Asp Leu Asn Met Tyr Lys Ser Glu Tyr Phe Ile Lys Gln Ile
165 170 175

Leu Leu Asn Leu Gly Val Ile Phe Phe Phe Thr Leu Ser Leu Ile Thr
180 185 190

Cys Ile Phe Leu Ile Ile Ser Leu Trp Arg His Asn Arg Gln Met Gln
195 200 205

Ser Asn Val Thr Gly Leu Arg Asp Ser Asn Thr Glu Ala His Val Lys
210 215 220

Ala Met Lys Val Leu Ile Ser Phe Ile Ile Leu Phe Ile Leu Tyr Phe
225 230 235 240

Ile Gly Met Ala Ile Glu Ile Ser Cys Phe Thr Val Arg Glu Asn Lys
245 250 255

Leu Leu Leu Met Phe Gly Met Thr Thr Thr Ala Ile Tyr Pro Trp Gly
260 265 270

His Ser Phe Ile Leu Ile Leu Gly Asn Ser Lys Leu Lys Gln Ala Ser
275 280 285

Leu Arg Val Leu Gln Gln Leu Lys Cys Cys Glu Lys Arg Lys Asn Leu
290 295 300

Arg Val Thr
305

<210> 38
<211> 921
<212> DNA
<213> Homo sapiens

<400> 38
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tctacgattg gctttattct caccggctta gctatttcaa gaatttttct gatatggata 180

ataattacag atggatttat acagatattc tctccaaata tatatgcctc cggtaaccta 240

attgaatata ttagttactt ttgggtaatt ggtaatcaat caagtatgtg gtttgccacc 300

agcctcagca tcttctattt cctgaagata gcaaattttt ccaactacat atttctctgg 360

ttgaagagca gaacaaatat ggttcttccc ttcatgatag tattcttact tatttcatcg 420

ttacttaatt ttgcatacat tgcgaagatt cttaatgatt ataaaatgaa gaatgacaca 480

gtctgggatc tcaacatgta taaaagtgaa tactttatta aacagatttt gctaaatctg 540

ggagtcattt tcttctttac actatcccta attacatgta ttttttaat catttccctt 600

tggagacaca acaggcagat gcaatcgaat gtgacaggat tgagagactc caacacagaa 660

gctcatgtga aggcaatgaa agttttgata tctttcatca tcctctttat cttgtatttt 720

ataggcatgg ccatagaaat atcatgtttt actgtgcgag aaaacaaact gctgcttatg 780

tttggaatga caaccacagc catctatccc tggggtcact catttatctt aattctagga 840

aacagcaagc taaagcaagc ctctttgagg gtactgcagc aattgaagtg ctgtgagaaa 900

aggaaaaatc tcagagtcac a 921

<210> 39
<211> 303
<212> PRT
<213> Homo sapiens

<400> 39

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Met Glu Ser Ala Leu Pro Ser Ile Phe Thr Leu Val Ile Ile Ala Glu
1               5                   10                  15

Phe Ile Ile Gly Asn Leu Ser Asn Gly Phe Ile Val Leu Ile Asn Cys
            20                  25                  30

Ile Asp Trp Val Ser Lys Arg Glu Leu Ser Ser Val Asp Lys Leu Leu
        35                  40                  45

Ile Ile Leu Ala Ile Ser Arg Ile Gly Leu Ile Trp Glu Ile Leu Val
    50                  55                  60

Ser Trp Phe Leu Ala Leu His Tyr Leu Ala Ile Phe Val Ser Gly Thr
65                  70                  75                  80

Gly Leu Arg Ile Met Ile Phe Ser Trp Ile Val Ser Asn His Phe Asn
                85                  90                  95

Leu Trp Leu Ala Thr Ile Phe Ser Ile Phe Tyr Leu Leu Lys Ile Ala
            100                 105                 110

Ser Phe Ser Ser Pro Ala Phe Leu Tyr Leu Lys Trp Arg Val Asn Lys
        115                 120                 125

Val Ile Leu Met Ile Leu Leu Gly Thr Leu Val Phe Leu Phe Leu Asn
    130                 135                 140

Leu Ile Gln Ile Asn Met His Ile Lys Asp Trp Leu Asp Arg Tyr Glu
145                 150                 155                 160

Arg Asn Thr Thr Trp Asn Phe Ser Met Ser Asp Phe Glu Thr Phe Ser
                165                 170                 175

Val Ser Val Lys Phe Thr Met Thr Met Phe Ser Leu Thr Pro Phe Thr
            180                 185                 190

Val Ala Phe Ile Ser Phe Leu Leu Leu Ile Phe Ser Leu Gln Lys His
        195                 200                 205

Leu Gln Lys Met Gln Leu Asn Tyr Lys Gly His Arg Asp Pro Arg Thr
    210                 215                 220

Lys Val His Thr Asn Ala Leu Lys Ile Val Ile Ser Phe Leu Leu Phe
225                 230                 235                 240
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Tyr Ala Ser Phe Phe Leu Cys Val Leu Ile Ser Trp Ile Ser Glu Leu
245 250 255

Tyr Gln Ser Thr Val Ile Tyr Met Leu Cys Glu Thr Ile Gly Val Phe
260 265 270

Ser Pro Ser Ser His Ser Phe Leu Leu Ile Leu Gly Asn Ala Lys Leu
275 280 285

Arg Gln Ala Phe Leu Leu Val Ala Ala Lys Val Trp Ala Lys Arg
290 295 300

<210> 40
<211> 909
<212> DNA
<213> Homo sapiens

<400> 40
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aatttgagca atggatttat agtactgatc aactgcattg actgggtcag taaaagagag 120

ctgtcctcag tcgataaact cctcattatc ttggcaatct ccagaattgg gctgatctgg 180

gaaatattag taagttggtt tttagctctg cattatctag ccatatttgt gtctggaaca 240

ggattaagaa ttatgatttt tagctggata gtttctaatc acttcaatct ctggcttgct 300

acaatcttca gcatctttta tttgctcaaa atagcgagtt tctctagccc tgcttttctc 360

tatttgaagt ggagagtaaa caaagtgatt ctgatgatac tgctaggaac cttggtcttc 420

ttatttttaa atctgataca aataaacatg catataaaag actggctgga ccgatatgaa 480

agaaacacaa cttggaattt cagtatgagt gactttgaaa cattttcagt gtcggtcaaa 540

ttcactatga ctatgttcag tctaacacca tttactgtgg ccttcatctc ttttctcctg 600

ttaattttct ccctgcagaa acatctccag aaaatgcaac tcaattacaa aggacacaga 660

gaccccagga ccaaggtcca tacaaatgcc ttgaaaattg tgatctcatt ccttttattc 720

tatgctagtt tctttctatg tgttctcata tcatggattt ctgagctgta tcagagcaca 780

gtgatctaca tgctttgtga gacgattgga gtcttctctc cttcaagcca ctcctttctt 840

ctgattctag gaaacgctaa gttaagacag gcctttcttt tggtggcagc taaggtatgg 900

gctaaacga 909

<210> 41
<211> 317
<212> PRT
<213> Homo sapiens

<400> 41

Met Gly Gly Val Ile Lys Ser Ile Phe Thr Phe Val Leu Ile Val Glu

1 5 10 15

Phe Ile Ile Gly Asn Leu Gly Asn Ser Phe Ile Ala Leu Val Asn Cys
20 25 30

Ile Asp Trp Val Lys Gly Arg Lys Ile Ser Ser Val Asp Arg Ile Leu
35 40 45

Thr Ala Leu Ala Ile Ser Arg Ile Ser Leu Val Trp Leu Ile Phe Gly
50 55 60

Ser Trp Cys Val Ser Val Phe Phe Pro Ala Leu Phe Ala Thr Glu Lys
65 70 75 80

Met Phe Arg Met Leu Thr Asn Ile Trp Thr Val Ile Asn His Phe Ser
85 90 95

Val Trp Leu Ala Thr Gly Leu Gly Thr Phe Tyr Phe Leu Lys Ile Ala
100 105 110

Asn Phe Ser Asn Ser Ile Phe Leu Tyr Leu Lys Trp Arg Val Lys Lys
115 120 125

Val Val Leu Val Leu Leu Leu Val Thr Ser Val Phe Leu Phe Leu Asn
130 135 140

Ile Ala Leu Ile Asn Ile His Ile Asn Ala Ser Ile Asn Gly Tyr Arg
145 150 155 160

Arg Asn Lys Thr Cys Ser Ser Asp Ser Ser Asn Phe Thr Arg Phe Ser
165 170 175

Ser Leu Ile Val Leu Thr Ser Thr Val Phe Ile Phe Ile Pro Phe Thr
180 185 190

Leu Ser Leu Ala Met Phe Leu Leu Leu Ile Phe Ser Met Trp Lys His
195 200 205

Arg Lys Lys Met Gln His Thr Val Lys Ile Ser Gly Asp Ala Ser Thr
210 215 220

Lys Ala His Arg Gly Val Lys Ser Val Ile Thr Phe Phe Leu Leu Tyr
225 230 235 240

Ala Ile Phe Ser Leu Ser Phe Phe Ile Ser Val Trp Thr Ser Glu Arg
245 250 255

Leu Glu Glu Asn Leu Ile Ile Leu Ser Gln Val Met Gly Met Ala Tyr
260 265 270

Pro Ser Cys His Ser Cys Val Leu Ile Leu Gly Asn Lys Lys Leu Arg
275 280 285

Gln Ala Ser Leu Ser Val Leu Leu Trp Leu Arg Tyr Met Phe Lys Asp
290 295 300

Gly Glu Pro Ser Gly His Lys Glu Phe Arg Glu Ser Ser
305 310 315

<210> 42
<211> 951
<212> DNA
<213> Homo sapiens

<400> 42

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atgggtggtg tcataaagag catatttaca ttcgttttaa ttgtggaatt tataattgga      60
aatttaggaa atagtttcat agcactggtg aactgtattg actgggtcaa gggaagaaag     120
atctcttcgg ttgatcggat cctcactgct ttggcaatct ctcgaattag cctggtttgg     180
ttaatattcg gaagctggtg tgtgtctgtg tttttcccag ctttatttgc cactgaaaaa     240
atgttcagaa tgcttactaa tatctggaca gtgatcaatc attttagtgt ctggttagct     300
acaggcctcg gtactttta ttttctcaag atagccaatt tttctaactc tatttttctc      360
tacctaaagt ggagagttaa aaaggtggtt ttggtgctgc ttcttgtgac ttcggtcttc     420
ttgtttttaa atattgcact gataaacatc catataaatg ccagtatcaa tggatacaga     480
agaaacaaga cttgcagttc tgattcaagt aactttacac gattttccag tcttattgta     540
ttaaccagca ctgtgttcat tttcataccc tttactttgt ccctggcaat gtttcttctc     600
ctcatcttct ccatgtggaa acatcgcaag aagatgcagc acactgtcaa aatatccgga     660
gacgccagca ccaaagccca cagaggagtt aaaagtgtga tcactttctt cctactctat     720
gccattttct ctctgtcttt tttcatatca gtttggacct ctgaaaggtt ggaggaaaat     780
ctaattattc tttcccaggt gatgggaatg gcttatcctt catgtcactc atgtgttctg     840
attcttggaa acaagaagct gagacaggcc tctctgtcag tgctactgtg gctgaggtac     900
atgttcaaag atggggagcc ctcaggtcac aaagaattta gagaatcatc t              951
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<210> 43
<211> 291
<212> PRT
<213> Homo sapiens

<400> 43

Met Ile Pro Ile Gln Leu Thr Val Phe Phe Met Ile Ile Tyr Val Leu

1 5 10 15

Glu Ser Leu Thr Ile Ile Val Gln Ser Ser Leu Ile Val Ala Val Leu
20 25 30

Gly Arg Glu Trp Leu Gln Val Arg Arg Leu Met Pro Val Asp Met Ile
35 40 45

Leu Ile Ser Leu Gly Ile Ser Arg Phe Cys Leu Gln Trp Ala Ser Met
50 55 60

Leu Asn Asn Phe Cys Ser Tyr Phe Asn Leu Asn Tyr Val Leu Cys Asn
65 70 75 80

Leu Thr Ile Thr Trp Glu Phe Phe Asn Ile Leu Thr Phe Trp Leu Asn
85 90 95

Ser Leu Leu Thr Val Phe Tyr Cys Ile Lys Val Ser Ser Phe Thr His
100 105 110

His Ile Phe Leu Trp Leu Arg Trp Arg Ile Leu Arg Leu Phe Pro Trp
115 120 125

Ile Leu Leu Gly Ser Leu Met Ile Thr Cys Val Thr Ile Ile Pro Ser
130 135 140

Ala Ile Gly Asn Tyr Ile Gln Ile Gln Leu Leu Thr Met Glu His Leu
145 150 155 160

Pro Arg Asn Ser Thr Val Thr Asp Lys Leu Glu Asn Phe His Gln Tyr
165 170 175

Gln Phe Gln Ala His Thr Val Ala Leu Val Ile Pro Phe Ile Leu Phe
180 185 190

Leu Ala Ser Thr Ile Phe Leu Met Ala Ser Leu Thr Lys Gln Ile Gln
195 200 205

His His Ser Thr Gly His Cys Asn Pro Ser Met Lys Ala His Phe Thr
210 215 220

Ala Leu Arg Ser Leu Ala Val Leu Phe Ile Val Phe Thr Ser Tyr Phe
225 230 235 240

Leu Thr Ile Leu Ile Thr Ile Ile Gly Thr Leu Phe Asp Lys Arg Cys
245 250 255

Trp Leu Trp Val Trp Glu Ala Phe Val Tyr Ala Phe Ile Leu Met His
260 265 270

Ser Thr Ser Leu Met Leu Ser Ser Pro Thr Leu Lys Arg Ile Leu Lys
275 280 285

Gly Lys Cys
290

<210> 44
<211> 873
<212> DNA
<213> Homo sapiens

<400> 44
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attattgtgc agagcagcct aattgttgca gtgctgggca gagaatggct gcaagtcaga 120

aggctgatgc ctgtggacat gattctcatc agcctgggca tctctcgctt ctgtctacag 180

tgggcatcaa tgctgaacaa tttttgctcc tattttaatt tgaattatgt actttgcaac 240

ttaacaatca cctgggaatt ttttaatatc cttacattct ggttaaacag cttgcttacc 300

gtgttctact gcatcaaggt ctcttctttc acccatcaca tctttctctg gctgaggtgg 360

agaattttga ggttgtttcc ctggatatta ctgggttctc tgatgattac ttgtgtaaca 420

atcatccctt cagctattgg gaattacatt caaattcagt tactcaccat ggagcatcta 480

ccaagaaaca gcactgtaac tgacaaactt gaaaattttc atcagtatca gttccaggct 540

catacagttg cattggttat tcctttcatc ctgttcctgg cctccaccat ctttctcatg 600

gcatcactga ccaagcagat acaacatcat agcactggtc actgcaatcc aagcatgaaa 660

gcgcacttca ctgccctgag gtcccttgcc gtcttattta ttgtgtttac ctcttacttt 720

ctaaccatac tcatcaccat tataggtact ctatttgata agagatgttg gttatgggtc 780

tgggaagctt ttgtctatgc tttcatctta atgcattcca cttcactgat gctgagcagc 840

cctacgttga aaaggattct aaagggaaag tgc 873

<210> 45
<211> 316
<212> PRT
<213> Homo sapiens

<400> 45

Met Met Gly Leu Thr Glu Gly Val Phe Leu Ile Leu Ser Gly Thr Gln
1 5 10 15

Phe Thr Leu Gly Ile Leu Val Asn Cys Phe Ile Glu Leu Val Asn Gly
20 25 30

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Ser Ser Trp Phe Lys Thr Lys Arg Met Ser Leu Ser Asp Phe Ile Ile
        35                  40                  45

Thr Thr Leu Ala Leu Leu Arg Ile Ile Leu Leu Cys Ile Ile Leu Thr
    50                  55                  60

Asp Ser Phe Leu Ile Glu Phe Ser Pro Asn Thr His Asp Ser Gly Ile
65                  70                  75                  80

Ile Met Gln Ile Ile Asp Val Ser Trp Thr Phe Thr Asn His Leu Ser
                85                  90                  95

Ile Trp Leu Ala Thr Cys Leu Gly Val Leu Tyr Cys Leu Lys Ile Ala
            100                 105                 110

Ser Phe Ser His Pro Thr Phe Leu Trp Leu Lys Trp Arg Val Ser Arg
        115                 120                 125

Val Met Val Trp Met Leu Leu Gly Ala Leu Leu Leu Ser Cys Gly Ser
    130                 135                 140

Thr Ala Ser Leu Ile Asn Glu Phe Lys Leu Tyr Ser Val Phe Arg Gly
145                 150                 155                 160

Ile Glu Ala Thr Arg Asn Val Thr Glu His Phe Arg Lys Lys Arg Ser
                165                 170                 175

Glu Tyr Tyr Leu Ile His Val Leu Gly Thr Leu Trp Tyr Leu Pro Pro
            180                 185                 190

Leu Ile Val Ser Leu Ala Ser Tyr Ser Leu Leu Ile Phe Ser Leu Gly
        195                 200                 205

Arg His Thr Arg Gln Met Leu Gln Asn Gly Thr Ser Ser Arg Asp Pro
    210                 215                 220

Thr Thr Glu Ala His Lys Arg Ala Ile Arg Ile Ile Leu Ser Phe Phe
225                 230                 235                 240

Phe Leu Phe Leu Leu Tyr Phe Leu Ala Phe Leu Ile Ala Ser Phe Gly
                245                 250                 255

Asn Phe Leu Pro Lys Thr Lys Met Ala Lys Met Ile Gly Glu Val Met
            260                 265                 270

Thr Met Phe Tyr Pro Ala Gly His Ser Phe Ile Leu Ile Leu Gly Asn
        275                 280                 285
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Ser Lys Leu Lys Gln Thr Phe Val Val Met Leu Arg Cys Glu Ser Gly
290 295 300

His Leu Lys Pro Gly Ser Lys Gly Pro Ile Phe Ser
305 310 315

<210> 46
<211> 948
<212> DNA
<213> Homo sapiens

<400> 46
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attctggtca attgtttcat tgagttggtc aatggtagca gctggttcaa gaccaagaga 120

atgtctttgt ctgacttcat catcaccacc ctggcactct tgaggatcat tctgctgtgt 180

attatcttga ctgatagttt tttaatagaa ttctctccca acacacatga ttcagggata 240

ataatgcaaa ttattgatgt ttcctggaca tttacaaacc atctgagcat ttggcttgcc 300

acctgtcttg gtgtcctcta ctgcctgaaa atcgccagtt tctctcaccc cacattcctc 360

tggctcaagt ggagagtttc tagggtgatg gtatggatgc tgttgggtgc actgctctta 420

tcctgtggta gtaccgcatc tctgatcaat gagtttaagc tctattctgt ctttaggggа 480

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tgtgagtctg gtcatctgaa gcctggatcc aagggaccca ttttctct 948

<210> 47
<211> 314
<212> PRT
<213> Homo sapiens

<400> 47

Met Ala Thr Glu Leu Asp Lys Ile Phe Leu Ile Leu Ala Ile Ala Glu
1 5 10 15

Phe Ile Ile Ser Met Leu Gly Asn Val Phe Ile Gly Leu Val Asn Cys
20 25 30

Ser Glu Gly Ile Lys Asn Gln Lys Val Phe Ser Ala Asp Phe Ile Leu
35 40 45

Thr Cys Leu Ala Ile Ser Thr Ile Gly Gln Leu Leu Val Ile Leu Phe
50 55 60

Asp Ser Phe Leu Val Gly Leu Ala Ser His Leu Tyr Thr Thr Tyr Arg
65 70 75 80

Leu Gly Lys Thr Val Ile Met Leu Trp His Met Thr Asn His Leu Thr
85 90 95

Thr Trp Leu Ala Thr Cys Leu Ser Ile Phe Tyr Phe Phe Lys Ile Ala
100 105 110

His Phe Pro His Ser Leu Phe Leu Trp Leu Arg Trp Arg Met Asn Gly
115 120 125

Met Ile Val Met Leu Leu Ile Leu Ser Leu Phe Leu Leu Ile Phe Asp
130 135 140

Ser Leu Val Leu Glu Ile Phe Ile Asp Ile Ser Leu Asn Ile Ile Asp
145 150 155 160

Lys Ser Asn Leu Thr Leu Tyr Leu Asp Glu Ser Lys Thr Leu Phe Asp
165 170 175

Lys Leu Ser Ile Leu Lys Thr Leu Leu Ser Leu Thr Ser Phe Ile Pro
180 185 190

Phe Ser Leu Ser Leu Thr Ser Leu Leu Phe Leu Phe Leu Ser Leu Val
195 200 205

Arg His Thr Arg Asn Leu Lys Leu Ser Ser Leu Gly Ser Arg Asp Ser
210 215 220

Ser Thr Glu Ala His Arg Arg Ala Met Lys Met Val Met Ser Phe Leu
225 230 235 240

Phe Leu Phe Ile Val His Phe Phe Ser Leu Gln Val Ala Asn Trp Ile
245 250 255

Phe Phe Met Leu Trp Asn Asn Lys Tyr Ile Lys Phe Val Met Leu Ala
260 265 270

Leu Asn Ala Phe Pro Ser Cys His Ser Phe Ile Leu Ile Leu Gly Asn
275 280 285

Ser Lys Leu Arg Gln Thr Ala Val Arg Leu Leu Trp His Leu Arg Asn
290 295 300

Tyr Thr Lys Thr Pro Asn Ala Leu Pro Leu
305 310

<210> 48
<211> 942
<212> DNA
<213> Homo sapiens

<400> 48
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atgctgggga atgtgttcat tggactggta aactgctctg aagggatcaa gaaccaaaag 120

gtcttctcag ctgacttcat cctcacctgc ttggctatct ccacaattgg acaactgttg 180

gtgatactgt ttgattcatt tctagtggga cttgcttcac atttatatac cacatataga 240

ctaggaaaaa ctgttattat gctttggcac atgactaatc acttgacaac ctggcttgcc 300

acctgcctaa gcattttcta tttctttaag atagcccact tcccccactc ccttttcctc 360

tggctgaggt ggaggatgaa cggaatgatt gttatgcttc ttatattgtc tttgttctta 420

ctgatttttg acagtttagt gctagaaata tttattgata tctcactcaa tataatagat 480

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ttaaaaactc ttctcagctt gaccagtttt atcccctttt ctctgtccct gacctccttg 600

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Ser Arg Phe Cys Leu Gln Ser Val Val Met Gly Lys Thr Ile Tyr Val
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Phe Leu His Pro Met Ala Phe Pro Tyr Asn Pro Val Leu Gln Phe Leu
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Ala Phe Gln Trp Asp Phe Leu Asn Ala Ala Thr Leu Trp Ser Ser Thr
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Trp Leu Ser Val Phe Tyr Cys Val Lys Ile Ala Thr Phe Thr His Pro
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Val Phe Phe Trp Leu Lys His Lys Leu Ser Gly Trp Leu Pro Trp Met
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Leu Phe Ser Ser Val Gly Leu Ser Ser Phe Thr Thr Ile Leu Phe Phe
145                 150                 155                 160

Ile Gly Asn His Arg Met Tyr Gln Asn Tyr Leu Arg Asn His Leu Gln
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Pro Trp Asn Val Thr Gly Asp Ser Ile Arg Ser Tyr Cys Glu Lys Phe
            180                 185                 190

Tyr Leu Phe Pro Leu Lys Met Ile Thr Trp Thr Met Pro Thr Ala Val
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Phe Phe Ile Cys Met Ile Leu Leu Ile Thr Ser Leu Gly Arg His Arg
    210                 215                 220

Lys Lys Ala Leu Leu Thr Thr Ser Gly Phe Arg Glu Pro Ser Val Gln
225                 230                 235                 240

Ala His Ile Lys Ala Leu Leu Ala Leu Leu Ser Phe Ala Met Leu Phe
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Ile Ser Tyr Phe Leu Ser Leu Val Phe Ser Ala Ala Gly Ile Phe Pro
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Pro Leu Asp Phe Lys Phe Trp Val Trp Glu Ser Val Ile Tyr Leu Cys
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Ala Val Leu Lys Ser Arg Arg Ser Ser Arg Cys Gly Thr Pro
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<223> Primer comprising NotI restriction site for PCR amplification of hTAS2R16

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